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(54) Title: INHIBITORS OF PRENYL TRANSFERASES

(57) Abstract

Compounds which inhibit prenyl transferases, particularly farnysyltransferase and geranylgeranyl transferase I, processes for preparing the compounds, pharmaceutical compositions containing the compounds, and methods of use.

INHIBITORS OF PRENYL TRANSFERASES

BACKGROUND OF THE INVENTION

1.   Field of the Invention

The present invention relates to novel
5   peptidomimetics and other compounds which are
useful as inhibitors of protein isoprenyl
transferases (particularly protein
farnesyltransferase and geranylgeranyltransferase)
and as anti-cancer drugs, to compositions
10   containing such compounds and to methods of use.

2.   Background Information

Ras proteins are plasma membrane- associated
GTPases that function as relay switches that
transduce biological information from
15   extracellular signals to the nucleus (29-31). In
normal cells Ras proteins cycle between the GDP-
(inactive) and GTP-(active) bound forms to
regulate proliferation and differentiation. The
mechanism by which extracellular signals, such as
20   epidermal and platelet derived growth factor (EGF
and PDGF), transduce their biological information
to the nucleus via Ras proteins has recently been
unraveled (29-31). Binding of the growth factors
to tyrosine kinase receptors results in
25   autophosphorylation of various tyrosines which
then bind src-homology 2 (SH2) domains of several
signaling proteins. One of these, a cytosolic
complex of GRB-2 and a ras exchanger (m-SOS-1), is
recruited by the tyrosine phosphorylated receptor
30   where mSOS-1 catalyzes the exchange of GDP for GTP
on Ras, hence activating it. GTP-bound Ras
recruits Raf, a serine/threonine kinase, to the
plasma membrane where it is activated. Raf
triggers a kinase cascade by phosphorylating
35   mitogen-activated protein (MAP)

kinase/extracellular-regulated protein kinase
(ERK) kinase (MEK) which in turn phosphorylates
MAP Kinase on threonine and tyrosine residues.
Activated MAP Kinase translocates to the nucleus
5   where it phosphorylates transcription factors
(31). Termination of this growth signal is
accomplished by hydrolysis of Ras-GTP to Ras-GDP.

        Ras oncogenes are the most frequently
identified activated oncogenes in human tumors (1-
10   3). In a large number of human cancers, Ras is
GTP-locked because of mutations in amino acids 12,
13, or 61 and the above Ras pathway no longer
requires an upstream growth signal and is
uninterrupted. As a consequence, enzymes in this
15   pathway such as Raf, MEK and MAP Kinase are
constitutively activated.

        In addition to its inability to hydrolyze
GTP, oncogenic Ras must be plasma membrane-bound
to cause malignant transformation (13). Ras is
20   posttranslationally modified by a lipid group,
farnesyl, which mediates its association with the
plasma membrane (10-14).

        Post-translational events leading to membrane
association of p21ras have previously been
25   disclosed (10-14). The p21ras proteins are first
made as pro-p21ras in the cytosol where they are
modified on cysteine 186 of their carboxyl
terminal sequence CA_1A_2X (C = cysteine, A_1 and A_2 =
isoleucine, leucine or valine and X = methionine
30   or serine) by the cholesterol biosynthesis
intermediate farnesyl pyrophosphate (FPP). This
farnesylation reaction is then followed by
peptidase removal of the A_1A_2X tripeptide and
carboxymethylation of the remaining cysteine. The
35   processed p21ras proteins associate with the inner

surface of the plasma membrane (10-14).

p21Ras farnesyltransferase, the enzyme responsible for catalyzing the transfer of farnesyl, a 15-carbon isoprenoid, from FPP to the
5   cysteine of the CA_1A_2X carboxyl terminus of p21ras, has been purified to homogeneity from rat brain (15,16). The enzyme is a heterodimer composed of α and β subunits of molecular weights 49 and 46 kDa, respectively (17). The β subunit has been
10   shown to bind p21ras (17). Because p21ras farnesylation and subsequent membrane association are required for p21ras transforming activity (13), it has been proposed that p21ras farnesyltransferase would be a useful anticancer
15   therapy target. Accordingly, an intensive search for inhibitors of the enzyme is underway (18-24, 33-44). Potential inhibitor candidates are CA_1A_2X tetrapeptides which have been shown to be farnesylated by p21ras farnesyltransferase and
20   appear to be potent inhibitors of this enzyme in vitro (15,18,21-24). Competition studies have demonstrated that CA_1A_2X peptides with the greatest inhibitory activity are those where A_1 and A_2 are hydrophobic peptides with charged or hydrophilic
25   residues in the central positions demonstrating very little inhibitory activity (18,21,23). A major drawback with the use of peptides as therapeutic agents is their low cellular uptake and their rapid inactivation by proteases.
30   The research efforts directed towards farnesyltransferase and the inhibition of its activity are further illustrated by the following patents or published patent applications:

U.S. 5,141,851
WO 91/16340
WO 92/18465
EPA 0456180 A1
5   EPA 0461869 A2
EPA 0512865 A2
EPA 0520823 A2
EPA 0523873 A1

        Of the above disclosures, EPA 0520823 A2 discloses
10   compounds which are useful in the inhibition of
    farnesyl-protein transferase and the farnesylation
    of the oncogene protein ras. The compounds of EPA
    0520823 A2 are illustrated by the formula:
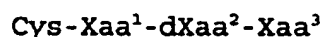
                        $Cys-Xaa^1-dXaa^2-Xaa^3$
15   or pharmaceutically acceptable salts thereof,
    wherein Cys is a cysteine amino acid;
    Xaa^1 is an amino acid in natural L-isomer form;
    dXaa2 is an amino acid in unnatural D-isomer form;
    and
20   Xaa^3 is an amino acid in natural L-isomer form.
        The preferred compounds are said to be
    CV(Dl)S and CV(Df)M, the amino acids being
    identified by conventional 3 letter and single
    letter abbreviations as follows:

25   Cysteine   Cys   C
    Glycine   Gly   G
    Isoleucine   Ile   I
    Leucine   Leu   L
    Lysine   Lys   K
30   Methionine   Met   M
    Phenylalanine   Phe   F
    Serine   Ser   S
    Threonine   Thr   T
    Valine   Val   V

                            - 4 -

EPA 0523873 A1 discloses a modification of
the compounds of EPA 0520823 A2 wherein Xaa³ is
phenylalanine or p-fluorophenylalanine.

EPA 0461869 describes compounds which inhibit

5   farnesylation of Ras protein of the formula:

Cys-Aaa¹-Aaa²-Xaa
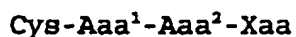
where Aaa¹ and Aaa² are aliphatic amino acids and
Xaa is an amino acid. The aliphatic amino acids
which are disclosed are Ala, Val, Leu and Ile.

10   Preferred compounds are those where Aaa¹ is Val,
Aaa² is Leu, Ile or Val and Xaa is Ser or Met.
Preferred specific compounds are:

Cys-Val-Leu-Ser

Cys-Val-Ile-Met

15   Cys-Val-Val-Met

U.S. patent 5,141,851 and WO 91/16340
disclose the purified farnesyl protein transferase
and certain peptide inhibitors therefor,
including, for example, CVIM, TKCVIM and

20   KKSKTKCVIM.

WO 92/18465 discloses certain farnesyl
compounds which inhibit the enzymatic methylation
of proteins including ras proteins.

EPA 0456180 A1 is directed to a

25   farnesylprotein transferase assay which can be
used to identify substances that block
farnesylation of ras oncogene gene products while
EPA 0512865 A2 discloses certain cyclic compounds
that are useful for lowering cholesterol and

30   inhibiting farnesylprotein transferase.

As will be evident from the foregoing, there
is a great deal of research effort directed
towards the development of inhibitors of
farnesyltransferase. However, there still remains

- 5 -

a need for improvements in this critically
important area.

An enzyme closely related to
farnesyltransferase, geranylgeranyltransferase I

5   (GGTase I), attaches the lipid geranylgeranyl to
the cysteine of the CAAX box of proteins where X
is leucine (49,69). FTase and GGTase I are α/β
heterodimers that share the α subunit (61,62).
Cross-linking experiments suggested that both

10   substrates (FPP and Ras CAAX) interact with the β
submit of FTase (17,63). Although GGTase I
prefers leucine at the X position, its substrate
specificity was shown to overlap with that of
FTase *in vitro* (64). Furthermore, GGTase I is

15   also able to transfer farnesyl to a leucine
terminating peptide (65).

Although CAAX peptides are potent competitive
inhibitors of FTase, rapid degradation and low
cellular uptake limit their use as therapeutic

20   agents. The stragegy of the present invention to
develop superior compounds for inhibiting FTase
and GGTase has been to replace several amino acids
in the CAAX motif by peptidemimics. The rationale
behind this strategy is based on the existance of

25   a hydrophobic pocket at the enzyme active site
that interacts with the hydrophobic "AA" dipeptide
of the carboxyl termini CAAX of Ras molecules. In
this regard, two very potent inhibitors of FTase
(i.e. Cys-3AMBA-Met and Cys-4ABA-Met) were

30   disclosed by us in an earlier U.S. patent
application. The peptidomimetic Cys-4ABA-Met
incorporates a hydrophobic/aromatic spacer (i.e.
4-aminobenzoic acid) between Cys and Met. The
present application discloses several derivatives

35   of Cys-4ABA-Met where positions 2 and 3 of 4-amino

benzoic acid were modified by several alkyl,
and/or aromatic groups, compounds that show great
promise of ability to <u>selectively</u> antagonize RAS-
dependent signaling and to selectively inhibit the
5   growth of human tumors with aberrant Ras function.
        Of the four types of Ras proteins (H-, N-,
K4A-, and K4B-Ras) expressed by mammalian cells,
K4B-Ras (also called K-Ras4B) is the most
frequently mutated form of Ras in human cancers
10   (1,3). Although several laboratories have
demonstrated potent inhibition of oncogenic H-Ras
processing and signaling (43,44), this disruption
has not been shown with K-Ras4B.   Previous
studies have targeted H-Ras and not K-Ras4B as a
15   target for the development of inhibitors. One
recent report indicates that K-Ras4B can be
geranylgeranylated *in vitro*, but with relatively
low efficiency; its K_m for GGTase I is 7 times
higher than its K_m for FTase (67). GGTase I CAAX-
20   based inhibitors that can block
geranylgeranylation processing have not been
reported.
        Recently, we have shown that a potent
inhibitor of FTase disrupts K-Ras4B processing but
25   only at very high concentrations that also
inhibited the processing of geranylgeranylated
proteins (66). This suggested that K-Ras4B may be
geranylgeranylated, and that therefore inhibitors
targeted at GGTase I would be effective in
30   disrupting oncogenic K-Ras4B processing and
signalling, and in treatment of cancers which were
related to this form of Ras.

Summary of the Invention

In accordance with the present invention there are compounds of the formula (A-L):

(A) (B) (C)

(D), (E) (F)

(G) (H) (I)

(J) (K) or (L)
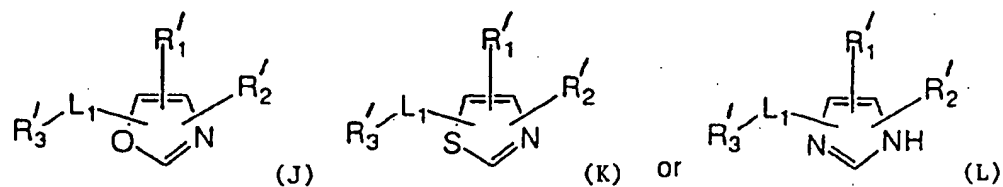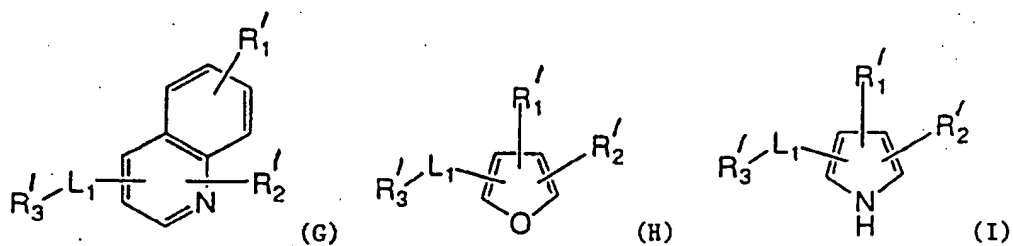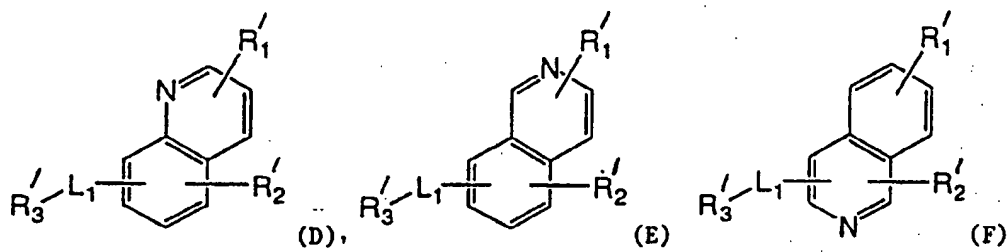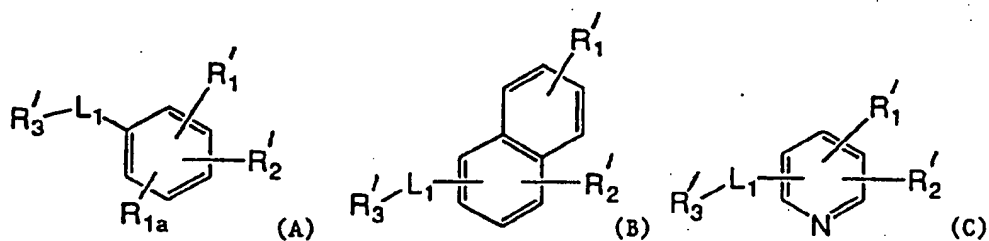
wherein R_1' is

i)   hydrogen;

ii) lower alkyl;

iii) alkenyl;

5   iv) alkoxy;

v)   thioalkoxy;

vi) halo;

vii) haloalkyl;

viii) aryl-L_2-, wherein L_2 is absent, -CH_2-, -

10   CH_2CH_2-, -$CH(CH_3)$-, -O-, -$S(O)_q$ wherein q is 0, 1,

or 2, -$N(R')$- wherein R' is hydrogen or lower

alkyl, or -$C(O)$- and aryl is selected from the

group consisting of phenyl, naphthyl,

tetrahydronaphthyl, indanyl and indenyl and the

15   aryl group is unsubstituted or substituted; or

ix) heterocyclic-L_3- wherein L_3 is absent, -CH_2-,

-CH_2CH_2-, -$CH(CH_3)$-, -O-, -$S(O)_q$ wherein q is 0, 1

or 2, -$N(R')$- wherein R' is hydrogen or

loweralkyl, or -$C(O)$- and heterocyclic is a

20   monocyclic heterocyclic wherein the heterocyclic

is unsubstituted or substituted with one, two, or

three substituents independently selected from the

group consisting of loweralkyl, hydroxy,

hydroxyalkyl, halo, nitro, oxo (=O), amino, N-

25   protected amino, alkoxy, thioalkoxy and haloalkyl;

R_{1a} is hydrogen or lower alkyl;

R_2' is

i)

30   wherein R_{12a} is hydrogen, loweralkyl or -$C(O)O$-R_{13},

wherein R_{13} is hydrogen or a carboxy-protecting

group and R_{12b} is hydrogen or loweralkyl, with the proviso that R_{12a} and R_{12b} are not both hydrogen,

ii) $-C(O)NH-CH(R_{14})-C(O)OR_{15}$ wherein R_{14} is
a) loweralkyl,
b) cycloalkyl,
c) cycloalkylalkyl,
d) alkoxyalkyl,
e) thioalkoxyalkyl,
f) hydroxyalkyl,
g) aminoalkyl,
h) carboxyalkyl,
i) alkoxycarbonylalkyl,
j) arylalkyl or
k) alkylsulfonylalkyl and

R_{15} is hydrogen or a carboxy-protecting group or

iii)

$$-C(O)-HN-\overset{\overset{\displaystyle O}{\|}}{\underset{\underset{\displaystyle (CH_2)_{1-3}}{\diagdown}}{\diagup}}O \quad ;$$

R_3' is

$$R_0-CH_2-CH-\overset{\overset{\displaystyle A}{\|}}{\underset{\underset{\displaystyle NH_2}{|}}{C}}-$$

where

A represents O or 2H, and

R_0 represents SH, NH_2, or $C_xH_y-SO_2-NH-$, wherein C_xH_y is a straight chain saturated or unsaturated hydrocarbon, with x being between 1 and 20 and y between 3 and 41, inclusive; and

- 10 -

L_1 is -NH-;

or pharmaceutically acceptable salts or prodrugs
thereof.

5   An important embodiment of the present
invention is based on the finding that a novel
group of peptidomimetics as represented by Formula
(I) have a high inhibitory potency against human
tumor p21ras farnesyltransferase and inhibit tumor
growth of human carcinomas:

10   CβX   (I)

where

C stands for the cysteine radical, or for the
reduced form of the cysteine radical (R-2-amino-3-
mercaptopropyl amine); β is the radical of a non-
15   peptide aminoalkyl- or amino-substituted phenyl
carboxylic acid; and X is the radical of an amino
acid, preferably Met. Any other natural or
synthetic amino acid can also be used at this
position. The invention also includes
20   pharmaceutically acceptable salts and prodrugs of
Formula (I).

A particularly preferred compound in this
regard is:
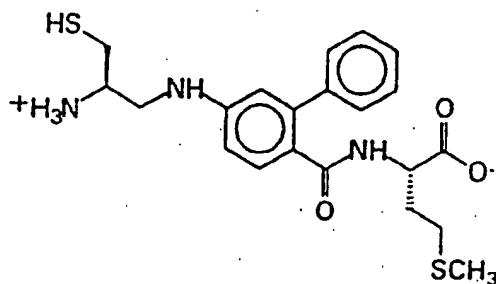
In this compound the cysteine radical is in the
reduced form and the spacer group is 2-phenyl-4-
aminobenzoic acid.

Another preferred compound of the invention

5   is:
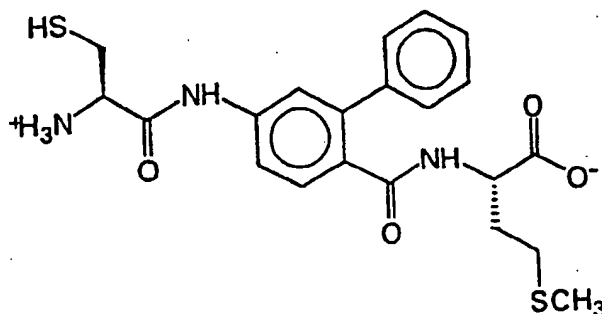
The compounds of Formula (I) are different
from the prior art farnesyltransferase inhibitors
in that they do not include separate peptide amino

10   acids A_1, A_2 as in prior art inhibitors represented
by the formula CA_1A_2X. The present compounds are
consequently free from peptidic amide bonds.

It is also to be noted that the present
compounds are not farnesylated by the enzyme.

15   They are, therefore, true inhibitors, not just
alternative substrates. This may explain the high
inhibitory action of the present compounds
relative to their parent compounds which are
farnesylated.

20   A further important feature of the invention
is the provision of the compounds of Formula (I)
in the form of pro-drugs. Broadly speaking, this
is accomplished by functionalizing the terminal
end groups (amino, cysteine sulfur and carboxy

25   groups) of the compounds with hydrophobic, enzyme-
sensitive moieties which serve to increase the
plasma membrane permeability and cellular uptake
of the compounds and consequently their efficiency
in inhibiting tumor cell growth. In addition,

- 12 -

prodrugs for amino and cysteine sulfur groups can
include loweralkycarbonyl, arylcarbony,
arylalkylcarbony, alkoxycarbonyl, aryloxycarbonyl,
cycloalkylcarbonyk, cycloalkoxycarbonyl, and other
5   groups well known to those skilled in the art.

In this regard, a particularly preferred
compound of the invention is the methylester form
of FTI-276, which is illustrated in Figure 1A.
The above-mentioned pro-drug aspect of the
10   invention is applicable not only to the compounds
of the invention but also to prior peptide
inhibitors CA_1A_2X as well as any other peptide with
potential for biological uses for the purpose of
improving the overall effectiveness of such
15   compounds, as hereinafter described.

A further modification involves the provision
of CA_1A_2X tetrapeptides or $C\beta X$ peptidomimetics
which have been modified by functionalizing the
sulfhydryl group of the cysteine C with an alkyl
20   phosphonate substituent, as hereinafter described.

Another important embodiment of the invention
contemplates replacing the A_1A_2X portion of the
CA_1A_2X tetrapeptide inhibitors with a non-amino
acid component while retaining the desired
25   farnesyltransferase inhibiting activity. These
compounds may be illustrated by Formula (II):

            CΔ (II)

where C is cysteine or reduced cysteine and Δ
represents an aryl or heterocyclic substituent
30   such as 3-aminomethyl-biphenyl-3'-carboxylic acid,
which does not include a peptide amino acid but
corresponds essentially in size with A_1A_2X, as
hereinafter described. The invention also
includes pharmaceutically acceptable salts and
35   prodrugs of Formula (II).

The invention also includes compounds in which further substitutions have been made at the cysteine position. These compounds comprise free cysteine thiol and/or terminal amino groups at one

5   end and include a carboxylic acid or carboxylate group at the other end, the carboxylic acid or carboxylate group being separated from the cysteine thiol and/or terminal amino group by a hydrophobic spacer moiety which is free from any

10   linking amido group as in prior CAAX mimetics. As with other compounds of the invention, these compounds are not subject to proteolytic degradation inside cells while retaining the structural features required for FTase inhibition.

15   The compounds selectively inhibit FTase both _in vitro_ and _in vivo_ and offer a number of other advantages over prior CAAX peptide mimetics.

Compounds of this embodiment may be illustrated by the formula:

20   C^0B (III)

where C^0 is

$$R_0-CH_2-CH-\overset{\overset{A}{\|}}{C}-$$
$$\underset{\underset{NH_2}{|}}{}$$

25

A represents O or 2H, and

R_0 represents SH, NH_2, or $C_xH_y-SO_2-NH-$, wherein C_xH_y is a straight chain saturated or unsaturated

30   hydrocarbon, with x being between 1 and 20 and y between 3 and 41, inclusive; and

B stands for -NHR, where R is an aryl group. The invention also includes pharmaceutically acceptable salts and prodrugs of Formula (III).

35   In one preferred embodiment of the invention, R is a biphenyl substituted with one or more -COOH

groups and/or lower alkyl, e.g., methyl, as
represented by the formula:
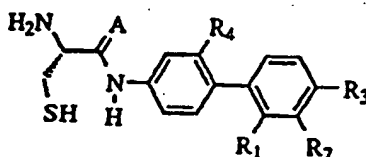
where R_1 and R_3 represent H or COOH; R_2 represents

5   H, COOH, CH_3, or $COOCH_3$; R_4 represents H or OCH_3;
and A represents 2H or O. This formula represents
a series of 4-amino-3'-carboxybiphenyl derivatives
which mimic the Val-Ile-Met tripeptide but have
restricted conformational flexibility. Reduction

10   of the cysteine amide bond (where A is H,H)
provides a completely non-peptidic Ras CAAX
mimetic.

        Preferably, R is a biphenyl group with a -
COOH substitution in the 3'- or 4'-position, most

15   preferably the 3'-position, with respect to the
NH-aryl group. The -COOH substituent may appear
as such or in pharmaceutically acceptable salt or
ester form, e.g., as the alkali metal salt or
methyl ester.

20   The features of the invention are illustrated
herein by reference to the CAAX tetrapeptide known
as CVIM (see EP 0461869 and U.S. Patent 5,141,851)
and C-4ABA-M. These compounds are, respectively,
Cys-Val-Ile-Met and Cys-4 aminobenzoic acid-Met

25   where Cys is the cysteine radical and Met is the
methionine radical.

        A preferred non-peptide CAAX mimetic of the
invention is reduced cys-4-amino-3'-
biphenylcarboxylate identified as 4 in Figure 12,

which is also designated FTI-265. This derivative
contains no amide bonds and thus is a true non-
peptide mimic of the CAAX tetrapeptide.

5   The compounds of the invention may be used in
the carboxylic acid form or as pharmaceutically
acceptable salts or esters thereof. Lower alkyl
esters are preferred although other ester forms,
e.g., phenyl esters, may also be used.

        It is also an object of the present invention
10   to provide a CAAX peptidomimetic that inhibits
GGTase I.

        Accordingly, it is an object of the present
invention to provide a substance and means of
disrupting oncogenic K-Ras4B processing and
15   signaling that affects geranylgeranylation and/or
farnesylation processing.

        It is a further object of the invention to
provide a pharmaceutical composition for treating
cancer which is responsive to geranylgeranyl
20   transferase inhibitors, such as, but not limited
to, pancreatic and colon cancer.

        The latter objects are accomplished by
replacing the central "AA" of CAAX tetrapeptides
by a hydrophobic spacer and incorporating a
25   leucine or isoleucine residue in the C-terminal
position to optimize recognition by GGTase I.
Additionally, the cysteine moiety may be replaced
by reduced cysteine, or by other functional groups
as hereinafter disclosed.

30   An important embodiment of the present
invention is based on the finding that a novel
group of peptidomimetics as represented by Formula
(IV) have a high inhibitory potency against
geranylgeranyl transferase and disrupt oncogenic
35   K-Ras4B processing and signalling:

                        CβL   (IV)

where

C stands for the cysteine radical, or for the
reduced form of the cysteine radical (R-2-amino-3-
mercaptopropyl amine); β is the radical of a non-
5   peptide aminoalkyl- or amino-substituted phenyl
carboxylic acid; and L is the radical of leucine
or isoleucine. The invention also includes
pharmaceutically acceptable salts and prodrugs of
the compounds of Formula (IV).

10   Preferred compounds of this embodiment are
derivatives of Cys-4ABA-Leu which are substituted
at the 2 and/or 3 positions of the phenyl ring of
4-aminobenzoic acid (4ABA). The substitutions at
these positions include, but are not limited to
15   alkyl, alkoxy and aryl (particularly to straight
chain or branched groups of 1-10 carbons of the
aforementioned) and naphthyl, heterocyclic rings
and heteroaromatic rings.

         A particularly preferred compound of this
20   aspect of the invention, GGTI-287, is illustrated
in Figure 17. In this compound the cysteine
radical is in the reduced form and the spacer
group is 2-phenyl-4-aminobenzoic acid. Another
preferred compound, also shown in Figure 17, is
25   GGTI-297, which contains the spacer group 2-
naphthyl-4-aminobenzoic acid.   Other spacer
groups which will be readily evident as useful are
described herein in connection with farnesyl-
transferase inhibitors.

30   A further important feature of the invention
is the provision of the compounds of the invention
in the form of pro-drugs. By "pro-drug" is meant
a compound to which in vivo modification occurs to
produce the active compound. Such compounds may,
35   for example, be more readily delivered to their
sites of action as pro-drugs. Broadly speaking,
the pro-drugs of the instant invention are

produced by functionalizing the terminal end
groups (amino, cysteine sulfur and carboxy groups)
of the compounds with hydrophobic, enzyme-
sensitive moieties which serve to increase the
5   plasma membrane permeability and cellular uptake
of the compounds and consequently their efficiency
in inhibiting tumor cell growth.

In this regard, a particularly preferred
compound of the invention is the methylester form
10   of GGTI-287, GGTI-286, also illustrated in Figure
17.

The compounds of the invention may be used in
the same manner as prior CAAX tetrapeptide
inhibitors to inhibit p21ras farnesyltransferase
15   or geranylgeranyl transferase in any host
containing these enzymes. This includes both _in_
vitro and _in vivo_ use. Compounds which inhibit
farnesyltransferase, notably human tumor p21ras
farnesyltransferase, and consequently inhibit the
20   farnesylation of the oncogene protein Ras, may be
used in the treatment of cancer or cancer cells.
It is noted that many human cancers have activated
ras and, as typical of such cancers, there may be
mentioned colorectal carcinoma, myeloid leukemias,
25   exocrine pancreatic carcinoma and the like.
Likewise, compounds which inhibit geranylgeranyl
transferase may be used in the treatment of cancer
which is related to K-Ras4B.

The compounds of the invention may be used in
30   pharmaceutical compositions of conventional form
suitable for oral, subcutaneous, intravenous,
intraperitoneal or intramuscular administration to
a mammal or host. This includes, for example,
tablets or capsules, sterile solutions or
35   suspensions comprising one or more compounds of
the invention with a pharmaceutically acceptable

carrier and with or without other additives.
Typical carriers for tablet or capsule use
include, for example, lactose or corn starch. For
oral compositions, aqueous suspensions may be used
5   with conventional suspending agents, flavoring
agents and the like.

The amount of inhibitor administered to
obtain the desired inhibitory effect will vary but
can be readily determined. It is expected that
10   the compounds of the present invention will be
administered to humans or other mammals as
pharmaceutical or chemotherapeutic agents in
dosages of .1 to 1000 mg/kg body weight,
preferably 1 to 500 mg/kg body weight and most
15   preferably 10-50 mg/kg body weight. The required
dose for a given individual or disease will vary,
but can be determined by ordinary skilled
practitioners using routine methods. The
compounds may be administered via methods well
20   known in the pharmaceutical and medical arts,
which include, but are not limited to oral,
parenteral, topical, and respiratory (inhalation)
routes. Pharmaceutical preparations may contain
suitable carriers or diluents. Means of
25   determining suitable carriers and diluents are
well known in the pharmaceutical arts.

The term "carboxy protecting group", as used
herein, refers to a carboxylic acid protecting
ester group employed to block or protect the
30   carboxylic acid functionally while the reactions
involving other functional sites of the compound
are carried out. Carboxy protecting groups are
disclosed in Greene, "Protective Groups in Organic
Synthesis", pp. 152-186 (1981), which is hereby
35   incorporated herein by reference. In addition, a
carboxy protecting group can be used as a prodrug
whereby the carboxy protecting group can be

- 19 -

readily cleaved *in vivo*, for example by enzymatic
hydrolysis, to release the biologically active
parent. A comprehensive discussion of the prodrug
concept is provided by T. Higuchi and V. Stella in
5   "Prodrugs as Novel Delivery Systems", vol. 14 of
the ACS Symposium Series, American Chemical
Society (1975), which is hereby incorporated by
reference. Such carboxy protecting groups are
will known to those skilled in the art, having
10   been extensively used in the protection of
carboxyl groups in the penicillin and
cephalosporin fields, as described in U.S. Pat.
No. 3,840,556 and 3,719,667, the disclosures of
which are hereby incorporated by reference.
15   Examples of esters useful as prodrugs for
compounds containing carboxyl groups can be found
on pages 14-21 of "Bioreversible Carriers in Drug
Design: Theory and Application", edited by E.B.
Roche, Permagon Press, New York, (1987) which is
20   hereby incorporated by reference. Representative
carboxy protecting groups are C1 to C8 loweralkyl
(e.g. methyl, ethyl or tertiary butyl and the
like); arylalkyl, for example, phenethyl or benzyl
and substituted drivatives thereof, for example 5-
25   indanyl and the like; dialkylaminoalkyl (e.g.
dimethylaminoethyl and the like); alkanoyloxyalkyl
groups such as acetoxymethol, butyryloxymethyl,
valeryloxymethyl, isobutyryloxymethyl,
isovaleryloxymethyl, 1-(propionyloxy)-1-ethyl, 1-
30   (pivaloyloxyl)-1-ethyl, 1-methyl-1-(propionyloxy)-
1-ethyl, pivaloyloxymethyl, propionyloxymethyl and
the like; cycloalkanoyloxyalkyl groups such as
cyclopropylcarbonyloxymethyl,
cyclobutylcarbonyloxymethyl,
35   cyclopentylcarbonyloxymethyl,
cyclohexylcarbonyloxymethyl and the like;
aroyloxyalkyl, such as benzoyloxymethyl,

benzoyloxyethyl and the like;
arylalkylcarbonyloxyalkyl, such as
benzylcarbonyloxymethyl, 2-benzylcarbonyloxyethyl
and the like; alkoxycarbonylalkyl or

5   cycloalkyloxycarbonylalkyl, such as
methoxycarbonylmethyl,
cyclohexyloxycarbonylmethyl, 1-methoxycarbonyl-1-
ethyl, and the like; alkoxycarbonyloxyalkyl or
cycloalkyloxycarbonylalkyl, such as

10   methoxycarbonyloxymethyl, t-
butyloxycarbonyloxymethyl, 1-ethoxycarbonyloxy-1-
ethyl, 1-cyclohexyloxycarbonyloxy-1-ethyl and the
like; aryloxycarbonyloxyalkyl, such as 2-
(phenoxycarbonyloxy)ethyl, 2-(5-

15   indanyloxycarbonyloxy)ethyl and the like;
alkoxyalkylcarbonyloxyalkyl, such as 2-(1-methoxy-
2-methylpropan-2-oyloxy)ethyl and the like;
arylalkyloxycarbonyloxyalkyl, such as 2-
(benzyloxycarbonyloxy)ethyl and the like;

20   arylalkenyloxycarbonyloxyalkyl, such as 2-(3-
phenylpropen-2-yloxycarbonyloxy)ethyl and the
like; alkoxycarbonylaminoalkyl, such as t-
butyloxycarbonylaminomethyl and the like;
alkylaminocarbonylaminoalkyl, such as

25   methylaminocarbonylaminomethyl and the like;
alkanoylaminoalkyl, such as acetylaminomethyl and
the like; heterocycliccarbonyloxyalkyl, such as 4-
methylpiperazinylcarbonyloxymethyl and the like;
dialkylaminocarbonylalkyl, such as

30   dimethylaminocarbonylmethyl,
diethylaminocarbonylmethyl and the like; (5-
(loweralkyl)-2-oxo-1,3-dioxolen-4-yl)alkyl, such
as (5-t-butyl-2-oxo-1,3-dioxolen-4-yl)methyl and
the like; and (5-phenyl-2-oxo-1,3-dioxolen-4-

35   yl)alkyl, such as (5-phenyl-2-oxo-1,3-dioxolen-4-
yl)methyl and the like.

Preferred carboxy-protected compounds of the invention are compounds wherein the protected carboxy group is a loweralkyl, cycloalkyl or arylalkyl ester, for example, methyl ester, ethyl ester, propyl ester, isopropyl ester, butyl ester, sec-butyl ester, isobutyl ester, amyl ester, isoamyl ester, octyl ester, cyclohexyl ester, phenylethyl ester and the like or an alkanoyloxyalkyl, cycloalkanoyloxyalkyl, aroyloxyalkyl or an arylalkylcarbonyloxyalkyl ester.

The term "N-protecting group" or "N-protected" as used herein refers to those groups intended to protect the N-terminus of an amino acid or peptide or to protect an amino group against undesirable reactions during synthetic procedures. Commonly used N-protecting groups are disclosed in Greene, "Protective Groups in Organic Synthesis," (John Wiley & Sons, New York (1981)), which is hereby incorporated herein by reference.

The term "alkanoyl" as used herein refers to $R_{29}C(O)-O-$ wherein R_{29} is a loweralkyl group.

The term "alkanoylaminoalkyl" as used herein refers to a loweralkyl radical to which is appended $R_{71}-NH-$ wherein R_{71} is an alkanoyl group.

The term "alkanoyloxy" as used herein refers to $R_{29}C(O)-O-$ wherein R_{29} is a loweralkyl group.

The term "alkanoyloxyalkyl" as used herein refers to a loweralkyl radical to which is appended an alkanoyloxy group.

The term "alkenyl" as used herein refers to a straight or branched chain hydrocarbon containing from 2 to 10 carbon atoms and also containing at least one carbon-carbon double bond. Examples of alkenyl include $-CH=CH_2$, $-CH_2CH=CH_2$, $-C(CH_3)=CH_2$, $-CH_2CH=CHCH_3$, and the like.

The term "alkenylene" as used herein refers to a divalent group derived from a straight or branched chain hydrocarbon containing from 2 to 10 carbon atoms and also containing at least one
5   carbon-carbon double bond. Examples of alkenylene include
$-CH=CH-$, $-CH_2CH=CH-$, $-C(CH_3)=CH-$, $-CH_2CH=CHCH_2-$, and the like.

The term "alkoxy" as used herein refers to
10     $R_{30}O-$ wherein R_{30} is loweralkyl as defined above. Representative examples of alkoxy groups include methoxy, ethoxy, t-butoxy and the like.

The term "alkoxyalkoxy" as used herein refers to $R_{31}O-R_{32}O-$ wherein R_{31} is loweralkyl as defined
15   above and R_{32} is an alkylene radical. Representative examples of alkoxyalkoxy groups include methoxymethoxy, ethoxymethoxy, t-butoxymethoxy and the like.

The term "alkoxyalkyl" as used herein refers
20   to an alkoxy group as previously defined appended to an alkyl group as previously defined. Examples of alkoxyalkyl include, but are not limited to, methoxymethyl, methoxyethyl, isopropoxymethyl and the like.

25   The term "alkoxyalkylcarbonyloxyalkyl" as used herein refers to a loweralkyl radical to which is appended $R_{66}-C(O)-O-$ wherein R_{66} is an alkoxyalkyl group.

The term "alkoxycarbonyl" as used herein
30   refers to an alkoxy group as previously defined appended to the parent molecular moiety through a carbonyl group. Examples of alkoxycarbonyl include methoxycarbonyl, ethoxycarbonyl, isopropoxycarbonyl and the like.

35   The term "alkoxycarbonylaklyl" as used herein refers to an alkoxylcarbonyl group as previously defined appended to a loweralkyl radical.

- 23 -

Examples of alkoxycarbonylaklyl include
methoxycarbonylmethyl, 2-ethoxycarbonylethyl and
the like.

5   The term "alkoxycarbonylaminoalkyl" as used
herein refers to a loweralkyl radical to which is
appended R_{69}-NH- wherein R_{69} is an alkoxycarbonyl
group.

          The term "alkoxycarbonyloxyalkyl" as used
herein refers to a loweralkyl radical to which is
10   appended R_{63}-O- wherein R_{63} is an alkoxycarbonyl
group.

          The term "alkylamino" as used herein refers
to R_{35}NH- wherein R_{35} is a loweralkyl group, for
example, methylamino, ethylamino, butylamino, and
15   the like.

          The term "alkylaminoalkyl" as used herein
refers a loweralkyl radical to which is appended
an alkylamino group.

          The term "alkylaminocarbonylaminoalkyl" as
20   used herein refers to a loweralkyl radical to
which is appended R_{70}-C(O)-NH- wherein R_{70} is an
alkylamino group.

          The term "alkylene" as used herein refers to
a divalent group derived from a straight or
25   branched saturated hydrocarbon having from 1 to 10
carbon atoms by the removal of two hydrogen atoms,
for example methylene, 1,2-ethylene, 1,1-ethylene,
1,3-propylene, 2,2-dimethylpropylene, and the
like.

30   The term "alkylsulfinyl" as used herein
refers to R_{33}S(O)- wherein R_{33} is a loweralkyl
group.

          The term "alkylsulfonyl" as used herein
refers to R_{34}S(O)$_2$- wherein R_{34} is a loweralkyl
35   group.

The term "alkylsulfonylalkyl" as used herein refers to a loweralkyl radical to which is appended an alkylsulfonyl group.

The term "alkynyl" as used herein refers to a straight or branched chain hydrocarbon containing from 2 to 10 carbon atoms and also containing at least one carbon-carbon triple bond. Examples of alkynyl include $-C\equiv CH$, $-CH_2C\equiv CH$, $-CH_2C\equiv CCH_3$, and the like.

The term "alkynylene" as used herein refers to a divalent group derived from a straight or branched chain hydrocarbon containing from 2 to 10 carbon atoms and also containing at least one carbon-carbon triple bond. Examples of alkynylene include

$-C\equiv C-$, $-CH_2C\equiv C-$, $-CH_2C\equiv CCH_2$, and the like.

The term "amino" as used herein refers to -NH_2.

The term "aminoalkyl" as used herein refers to a loweralkyl radical to which is appended an amino group.

The term "aroyloxyalkyl" as used herein refers to a loweralkyl radical to which is appended an aroyloxy group (i.e., $R_{61}-C(O)O-$ wherein R_{61} is an aryl group).

The term "aryl" as used herein refers to a mono- or bicyclic carbocyclic ring system having one or two aromatic rings including, but not limited to, phenyl, naphthyl, tetrahydronaphthyl, indanyl, indenyl and the like. Aryl groups (including bicyclic aryl groups) can be unsubstituted or substituted with one, two or three substituents independently selected from loweralkyl, haloalkyl, alkoxy, thioalkoxy, amino, alkylamino, dialkylamino, hydroxy, halo, mercapto, nitro, cyano, carboxaldehyde, carboxy, alkoxycarbonyl, haloalkyl-C(O)-NH-, haloalkenyl-C(O)-NH- and

- 25 -

carboxamide. In addition, substituted aryl groups include tetrafluorophenyl and pentafluorophenyl.

The term "arylalkenyl" as used herein refers to an alkenyl radical to which is appended an aryl
5   group.

The term "arylalkenyloxycarbonyloxyalkyl" as used herein refers to a loweralkyl radical to which is appended R_{68}-O-C(O)-O- wherein R_{68} is an arylalkenyl group.

10   The term "arylalkyl" as used herein refers to a loweralkyl radical to which is appended an aryl group. Representative arylalkyl groups include benzyl, phenylethyl, hydroxybenzyl, fluorobenzyl, fluorophenylethyl and the like.

15   The term "arylalkylcarbonyloxyalkyl" as used herein refers to a loweralkyl radical to which is appended an arylalkylcarbonyloxy group (i.e., R_{62}C(O)O- wherein R_{62} is an arylalkyl group).

The term "arylalkyloxycarbonyloxyalkyl" as
20   used herein refers to a loweralkyl radical to which is appended R_{67}-O-C(O)-O- wherein R_{67} is an arylalkyl group.

The term "aryloxyalkyl" as used herein refers to a loweralkyl radical to which is appended R_{65}-O-
25   wherein R_{65} is an aryl group.

The term "aryloxthioalkoxyalkyl" as used herein refers to a loweralkyl radical to which is appended R_{75}-S- wherein R_{75} is an aryloxyalkyl group.

30   The term "aryloxycarbonyalkyl" as used herein refers to a loweralkyl radical to which is appended R_{65}-O-C(O)-O- wherein R_{65} is an aryl group.

The term "arylsulfonyl" as used herein refers to R_{36}S(O)$_2$- wherein R_{36} is an aryl group.

35   The term "arylsulfonyloxy" as used herein refers to R_{37}S(O)$_2$O- wherein R_{37} is an aryl group.

The term "carboxyalkyl" as used herein refers
to a loweralkyl radical to which is appended a
carboxy (-COOH) group.

The term "carboxaldehyde" as used herein
refers to the group -C(O)H.

The term "carboxamide" as used herein refers
to the group -C(O)NH$_2$.

The term "cyanoalkyl" as used herein refers
to a loweralkyl radical to which is appended a
cyano (-CN) group.

The term "cycloalkanoylalkyl" as used herein
refers to a loweralkyl radical to which is
appended a cycloalkanoyl group (i.e., R$_{60}$-C(O)-
wherein R$_{60}$ is a cycloalkyl group).

The term "cycloalkanoyloxyalkyl" as used
herein refers to a loweralkyl radical to which is
appended a cycloalkanoyloxy group (i.e., R$_{60}$-C(O)O-
wherein R$_{60}$ is a cycloalkyl group).

The term "cycloalkenyl" as used herein refers
to an alicyclic group comprising from 3 to 10
carbon atoms and containing a carbon-carbon double
bond including, but not limited to, cyclopentenyl,
cyclohexenyl and the like.

The term "cycloalkyl" as used herein refers
to an alicyclic group comprising from 3 to 10
carbon atoms including, but not limited to,
cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl,
norbornyl, adamantyl and the like.

The term "cycloalkylalkyl" as used herein
refers to a loweralkyl radical to which is
appended a cycloalkyl group. Representative
examples of cycloalkylalkyl include
cyclopropylmethyl, cyclohexylmethyl, 2-
(cyclopropyl)ethyl, adamantylmethyl and the like.

The term "cycloalkyloxycarbonyloxyalkyl" as
used herein refers to a loweralkyl radical to

- 27 -

which is appended R_{64}-O-C(O)-O- wherein R_{64} is a cycloalkyl group.

The term "dialkoxyalkyl" as used herein refers to a loweralkyl radical to which is appended two alkoxy groups.

The term "dialkylamino" as used herein refers to $R_{38}R_{39}N$- wherein R_{38} and R_{39} are independently selected from loweralkyl, for example, dimethylamino, diethylamino, methyl propylamino, and the like.

The term "dialkylaminoalkyl" as used herein refers to a loweralkyl radical to which is appended a dialkylamino group.

The term "dialkyaminocarbonylalkyl" as used herein refers to a loweralkyl radical to which is appended R_{73}-C(O)- wherein R_{73} is a dialkylamino group.

The term "dioxoalkyl" as used herein refers to a loweralkyl radical which is substituted with two oxo (=O) groups.

The term "dithioalkoxyalkyl" as used herein refers to a loweralkyl radical to which is appended two thioalkoxy groups.

The term "halogen" or "halo" as used herein refers to I, Br, Cl or F.

The term "haloalkenyl" as used herein refers to an alkenyl radical, as defined above, bearing at least one halogen substituent.

The term "haloalkyl" as used herein refers to a lower alkyl radical, as defined above, bearing at least one halogen substituent, for example, chloromethyl, fluoroethyl or trifluoromethyl and the like.

The term "heterocyclic ring" or "heterocyclic" or "heterocycle" as used herein refers to a 5-, 6- or 7-membered ring containing one, two or three heteroatoms independently

selected from the group consisting of nitrogen,
oxygen and sulfur or a 5-membered ring containing
4 nitrogen atoms; and includes a 5-, 6- or 7-
membered ring containing one, two or three
5   nitrogen atoms; one oxygen atom; one sulfur atom;
one nitrogen and one sulfur atom; one nitrogen and
one oxygen atom; two oxygen atoms in non-adjacent
positions; one oxygen and one sulfur atom in non-
adjacent positions; two sulfur atoms in non-
10   adjacent positions; two sulfur atoms in adjacent
positions and one nitrogen atom; two adjacent
nitrogen atoms and one sulfur atom; two non-
adjacent nitrogen atoms and one sulfur atom; two
non-adjacent nitrogen atoms and one oxygen atom.
15   The 5-membered ring has 0-2 double bonds and the
6- and 7-membered rings have 0-3 double bonds.
The term "heterocyclic" also includes bicyclic,
tricyclic and tetracyclic groups in which any of
the above heterocyclic rings is fused to one or
20   two rings independently selected from the group
consisting of an aryl ring, a cyclohexane ring, a
cyclohexene ring, a cyclopentane ring, a
cyclopenene ring and another monocyclic
heterocyclic ring (for example, indolyl, quinolyl,
25   isoquinolyl, tetrahydroquinolyl, benzofuryl or
benzothienyl and the like). Heterocyclics
include: pyrrolyl, pyrrolinyl, pyrrolidinyl,
pyrazolyl, pyrazolinyl, pyrazolidinyl, imidazolyl,
imidazolinyl, imidazolidinyl, pyridyl,
30   piperidinyl, homopiperidinyl, pyrazinyl,
piperazinyl, pyrimidinyl, pyridazinyl, oxazolyl,
oxazolidinyl, isoxazolyl, isoxazolidinyl,
morpholinyl, thiomorpholinyl, thiazolyl,
thiazolidinyl, isothiazolyl, isothiazolidinyl,
35   indolyl, quinolinyl, isoquinolinyl,
benzimidazolyl, benzothiazolyl, benzoxazolyl,
furyl, thienyl, thiazolidinyl, isothiazolyl,

- 29 -

triazolyl, tetrazolyl, oxadiazolyl, thiadiazolyl,
pyrimidyl, tetrahydrofuranyl, dihydrofuranyl,
tetrahydrothienyl, dihydrothienyl, dihydroindolyl,
tetrahydroquinolyl, tetrahydroisoquinolyl,
pyranyl, dihydropyranyl, dithiazolyl, benzofuranyl
and benzothienyl. Heterocyclics also include
bridged bicyclic groups wherein a monocyclic
heterocyclic group is bridged by an alkylene
group, for example,
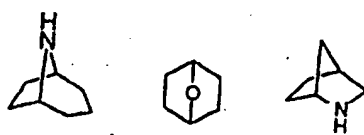
and the
like.

Heterocyclics also include compounds of the
formula
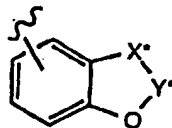
wherein X* is $-CH_2-$, $-CH_2O-$ or $-O-$ and Y* is $-C(O)-$
or $-(C(R'')_2)_v-$ wherein R'' is hydrogen or C_1-C_4-alkyl
and v is 1, 2 or 3 such as 1,3-benzodioxolyl, 1,4-
benzodioxanyl and the like.
Heterocyclics can be unsubstituted or
substituted with one, two or three substituents
independently selected from the group consisting
of
a)   hydroxy,
b)   -SH,
c)   halo,
d)   oxo (=O),
e)   thioxo (=S),
f)   amino,
g)   -NHOH,
h)   alkylamino,
i)   dialkylamino,

j)   alkoxy,

k)   alkoxyalkoxy.

l)   haloalkyl.

m)   hydroxyalkyl,

5   n)   alkoxyalkyl,

o)   cycloalkyl,

p)   cycloalkenyl,

q)   alkenyl,

r)   alkynyl,

10   s)   aryl,

t)   arylalkyl,

u)   -COOH,

v)   -SO$_3$H,

w)   loweralkyl,

15   x)   alkoxycarbonyl,

y)   -C(O)NH$_2$,

z)   -C(S)NH$_2$,

aa)   -C(=N-OH)NH$_2$,

bb)   loweralkyl-C(O)-,

20   cc)   loweralkyl-C(S)-,

dd)   formyl,

ee)   cyano, and

ff)   nitro.

The term "(heterocyclic)alkyl" as used herein

25   refers to a heterocyclic group as defined above
appended to a loweralkyl radical as defined above.
Examples of heterocyclic alkyl include 2-
pyridylmethyl, 4-pyridylmethyl, 4-quinolinylmethyl
and the like.

30   The term "heterocycliccarbonyloxyalkyl" as
used herein refers to a loweralkyl radical to
which is appended R$_{72}$-C(O)-O- wherein R$_{72}$ is a
heterocyclic group.

The term "hydroxyalkyl" as used herein refers

35   to a loweralkyl radical to which is appended an
hydroxy group.

The term "hydroxythioalkoxy" as used herein refers to $R_{51}S-$ wherein R_{51} is a hydroxyalkyl group.

The term "loweralkyl" as used herein refers to branched or straight chain alkyl groups

5   comprising one to ten carbon atoms, including methyl, ethyl, propyl, isopropyl, n-butyl, neopentyl and the like.

The term "N-protected alkylaminoalkyl" as used herein refers to an alkylaminoalkyl group

10   wherein the nitrogen is N-protected.

The term "oxoalkyloxy" as used herein refers to an alkoxy radical wherein the loweralkyl moiety is substituted with an oxo (=O) group.

The term "spiroalkyl" as used herein refers

15   to an alkylene diradical, both ends of which are bonded to the same carbon atom of the parent group to form a spirocyclic group.

The term "thioalkoxy" as used herein refers to $R_{52}S-$ wherein R_{52} is loweralkyl. Examples of

20   thioalkoxy include, but are not limited to, methylthio, ethylthio and the like.

The term "thioalkoxyalkyl" as used herein refers to a thioalkoxy group as previously defined appended to a loweralkyl group as previously

25   defined. Examples of thioalkoxyalkyl include thiomethoxymethyl, 2-thiomethoxyethyl and the like.

The present invention also relates to processes for preparing the compounds of formula

30   (1)-(Xll) and to the synthetic intermediates useful in such processes.

In a further aspect of the present invention are disclosed pharmaceutical compositions which comprise a compound of the present invention in

35   combination with a pharmaceutically acceptable carrier.

In yet another aspect of the present
invention are disclosed pharmaceutical
compositions which comprise a compound of the
present invention in combination with another
5   chemotherapeutic agent and a pharmaceutically
acceptable carrier.

In yet another aspect of the present
invention is disclosed a method for inhibiting
protein isoprenyl transferases (i.e., protein
10   farnesyltransferase and/or
geranylgeranyltransferase) in a human or lower
mammal, comprising administering to the patient a
therapeutically effective amount of a compound of
the invention.

15   In yet another aspect of the present
invention is disclosed a method for inhibiting
post-translational modification of the oncogenic
Ras protein by protein farnesyltransferase,
protein geranylgeranyltransferase or both.

20   In yet another aspect of the present
invention is disclosed a method for treatment of
conditions mediated by farnesylated or
geranylgeranylated proteins, for example,
treatment of Ras associated tumors in humans and
25   other mammals.

In yet another aspect of the present
invention is disclosed a method for inhibiting or
treating cancer in a human or lower mammal,
comprising administering to the patient a
30   therapeutically effective amount of a compound of
the invention alone or in combination with another
chemotherapeutic agent.

In yet another aspect of the present
invention is disclosed a method for treating or
35   preventing restenosis in a human or lower mammal,
comprising administering to the patient a

therapeutically effective amount of a compound of
the invention.

The compounds of the present invention can be
used in the form of pharmaceutically acceptable
5   salts derived from inorganic or organic acids.
These salts include but are not limited to the
following: acetate, adipate, alginate, citrate,
aspartate, benzoate, benzenesulfonate, bisulfate,
butyrate, camphorate, camphorsulfonate,
10   digluconate, cyclopentanepropionate,
dodeoylsulfate, ethanesulfonate, glucoheptanoate,
glycerophosphate, hemisulfate, heptanoate,
hexanoate, fumarate, hydrochloride, hydrobromide,
hydroiodide, 2-hydroxy-ethanesulfonate, lactate,
15   maleate, methanesulfonate, nicotinate, 2-
naphthalenesulfonate, oxalate, pamoate, pectinate,
persulfate, 3-phenylpropionate, picrate, pivalate,
propionate, succinate, tartrate, thiocyanate, p-
toluenesulfonate and undecanoate. Also, the basic
20   nitrogen-containing groups can be quaternized with
such agents as loweralky halides (such as methyl,
ethyl, propyl, and butyl chloride, bromides, and
iodides), dialkyl sulfates like dimethyl, diethyl,
dibutyl, and diamyl sulfates, long chain halides
25   such as decyl, lauryl, myristyl and stearyl
chlorides, bromides and iodides, aralkyl halides
like benzyl and phenethyl bromides, and others.
Water or oil-soluble or disperisble products are
thereby obtained.
30   Examples of acids which may be employed to
form pharmaceutically acceptable acid addition
sales include such inorganic acids as hydrochloric
acid, sulphuric acid and phosphoric acid and such
organic acids as oxalic acid, maleic acid,
35   succinic acid and citric acid.

Basic addition salts can be prepared *in situ*
during the final isolation and purification of the

compounds of formulas A-L, or separately by
reacting the carboxylic acid function with a
suitable base such as the hydroxide, carbonate or
bicarbonate of a pharmaceutically acceptable metal
5   cation or with ammonia, or an organic primary,
secondary or tertiary amine. Such
pharmaceutically acceptable salts include, but are
not limited to, cations based on the alkali and
alkaline earth metals, such as sodium, lithium,
10   potassium, calcium, magnesium, aluminum salts and
the likes, as well as nontoxic ammonium,
quaternary ammonium, and amine cations, including,
but not limited to ammonium, tetramethylammonium,
tetraethylammonium, methylamine, dimethylamine,
15   trimethylamine, triethylamine, ethylamine, and the
like. Other representative organic amines useful
for the formation of base addition salts include
diethylamine, ethylenediamine, ethanolamine,
diethanolamine, piperazine and the like.
20   Other features of the invention will also be
hereinafter apparent.

Brief Description of the Drawings
Figure 1: Ras CAAX peptidomimetics and
FTase/GGTase I activities
25   A.   Structures of CVIM, FTI-249, FTI-276 and FTI-
277. FTI-276 and FTI-277 were synthesized as
described in Examples 10 and 11. B. FTase and
GGTase I inhibition assays were carried out as
described in Example 12 by determining the ability
30   of FTI-276 to inhibit the transfer of farnesyl and
geranylgeranyl to recombinant H-Ras-CVLS and H-
Ras-CVLL, respectively. The data are
representative of at least three different
experiments.

Figure 2: Inhibition of Ras and Rap1A Processing
A.   H-RasF cells were treated with various
concentrations of FTI-277, lysed and the lysates
immunoblotted with anti-Ras or anti-Rap1A
5   antibodies as described in Example 13. B.
pZIPneo, H-RasF, H-RasGG, Raf and S186 cells were
treated with vehicle or FTI-277 (5 μM), lysed and
lysates immunoblotted by anti-Ras antibody. Data
is representative of 5 different experiments. The
10   cells were obtained from Dr. Channing Der,
University of North Carolina, Chapel Hill, North
Carolina.

Figure 3: Effects of FTI-277 on Ras/Raf
Association. pZIPneo, H-RasF, H-RasGG and S186
15   cells were treated with vehicle or FTI-277 (5 μM),
homogenized and the membrane (A) and cytosolic (B)
fractions were separated and immunoprecipitated by
an anti-Raf antibody. The immunoprecipitates were
then separated by SDS-PAGE and immunoblotted with
20   anti-RAS antibody as described in Example 14.
Data is representative of three different
experiments.

Figure 4: Effects of FTI-277 on Ras Nucleotide
Binding and Raf Kinase Activity
25   A: H-RasF cells were treated with vehicle or FTI-
277, lysed and the lysates immunoprecipitated with
anti-Ras antibody. The GTP and GDP were then
released from Ras and separated by TLC as
described in Example 15. B: pZIPneo and H-RasF
30   cells were treated with vehicle or FTI-277, lysed
and cells lysates immunoprecipitated with an anti-
Raf antibody. Raf kinase was assayed by using a
19-mer autophosphorylation peptide as substrate as
described in Example 16. Data are representative
35   of three different experiments.

Figure 5: Effect of FTI-277 on Oncogenic Activation of MAPK

A: H-RasF cells were treated with various concentrations of FTI-277, cells lysed and lysates
5   run on SDS-PAGE and immunoblotted with anti-MAPK antibody. B: pZIPneo, H-RasF, H-RasGG, Raf, and S186 cells were treated with vehicle of FTI-277 (5 μM), lysed and cells lysates processed as for A. Data are representative of two different
10   experiments.

Figure 6. FTI-276 inhibits selectively Ras processing and oncogenic Ras activation of MAP Kinase. NIH 3T3 cells transfected with empty vector (pZIPneo), oncogenic (GTP-locked)
15   farnesylated Ras (RasF), geranylgeranylated Ras (RasGG) or a transforming mutant of human Raf-1 were obtained from Channing Der and Adrienne Cox (University of North Carolina, Chapel Hill, NC, USA) (26,27). The cells were plated in DMEM/10%
20   CS (Dubelco's Modified Eagles Medium, 10% calf serum) on day one and treated with vehicle or FTI-270 (20 μM) on days 2 and 3. The cells were then harvested on day 4 and lysed in lysis buffer (30 mM HEPES, pH 7.5, 1% TX-100, 10% glycerol, 10 mM
25   NaCl, 5 mM MgCl$_2$, 25 mM NaF, 1 mM EGTA, 2 mM Na$_3$VO$_4$, 10 μg/ml Trypsin inhibitor, 25 μg/ml leupeptin, 10 μg/ml aprotinin, 2 mM PMSF). The lysate (35 μg) was electrophoresed on 15% SDS-PAGE, transferred to nitrocellulose membranes and
30   immunoblotted simultaneously with anti-Ras antibody Y13-238 (isolated from hybridomas purchased from ATCC, Rockville, MD) and an Anti-MAP kinase (erk2) antibody (UBI, Lake Placid, NY) as described previously (17, 22).

Figure 7. Antitumor efficacy of FTI-276 against
human lung carcinomas. Calu-1 (Panel A) and NCI-
H810 cells (Panel B) were purchased from ATCC and
grown in McCoy's 5A medium in 10% FBS (Fetal
5   Bovine Serum) and RPMI 1640 in 10% FBS,
respectively. The cells were harvested,
resuspended in PBS and injected s.c. into the
right and left flank of 8 week old female nude
mice (10^7 cells/flank). Nude mice (Harlan Sprague
10   Dawley, Indianapolis, Indiana) were maintained in
accordance with the Institutional Animal Care and
Use Committee (IACUC) procedures and guidelines.
On day 32 after s.c. implantation of tumors,
animals were dosed i.p. with 0.2 ml once daily for
15   36 days. Control animals (filled circles)
received a saline vehicle whereas treated animals
(open triangles) were injected with FTI-276 (50
mg/kg). The tumor volumes were determined by
measuring the length (l) and the width (w) and
20   calculating the volume ($V = (l) \times (w)^2/2$). Data
are presented as the average volume of eight
tumors in each group for each cell line.
Statistical significance between control and
treated groups were evaluated by using student t
25   test ($^*P<0.05$).

Figure 8. Antitumor Efficacy of FTI-276 and FTI-
277 in Human Lung Carcinoma (Calu-1) Cells.
Experimental procedure was the same as described
in Figure 7.

30   Figure 9. Inhibition of Tumor Growth in Ras
transformed cells by FTI-276 and FTI-277. Ras-
transformed NIH 3T3 cells were implanted
subcutaneously into nude mice, and daily
intraperitoneal injections with FTI-276 and FTI-

277 (50 mg/kg) were started when the tumors
reached 50 mm³.

5   **Figure 10. Inhibition of Tumor Growth in Raf
transformed cells by FTI-276 and FTI-277.** Raf-
transformed NIH 3T3 cells were implanted
subcutaneously into nude mice, and daily
intraperitoneal injections with FTI-276 and FTI-
277 (50 mg/kg) were started when the tumors
10   reached 50 mm³.

**Figure 11. Dose response: Antitumor efficacy and
Ras processing correlations.** A. Antitumor
efficacy was carried out as described in Fig. 3
except that animals were randomly assigned to four
15   groups each of 4 mice each (2 tumors per mouse).
Saline treated groups (circles); FTI-276 treated
groups: 10 mg/kg (squares), 50 mg/kg (upward
triangles), 100 mg/kg (downward triangles). B.
Ras processing was carried out 5 hours after the
20   last treatement on day 17. Tumors were extracted
from the animals, tissumized, and lysed in lysis
buffer as described in Fig. 1. Lysates (25 µg)
were electrophoresed on a 12.5% SDS-PAGE and
immunoblotted with anti-Ras antibody Y13-238 as
25   described previously. The blots were then
reprobed with anti-Rap1A antibody (Santa Cruze
Biotechnologies, Santa Cruz, California).

**Figure 12. Structures of CVIM, C-4ABA-M, reduced
C-4ABA-M, FTI-265 (4), FTI-271 (5), and FTI-261
30   (8).**

**Figure 13. Energy-minimized structural
conformations for CVIM and farnesyltransferase
inhibitor FTI-265.**

Figures 14A and B. Comparison of FTase and GGTase
I inhibition by FTI-265 and FTI-271.

Figure 15. Silica gel TLC relating to Ras CAAX
peptide and peptidomimetic farnesylation.

5   Figure 16. Ras and Rap1A processing in cells
using a compound according to the invention.

Figure 17.   CAAX peptidomimetic structures.
Structures of FTI-276/277, GGTI-287/286, and GGTI-
297.

10   Figure 18.   Disruption of H-Ras and Rap1A
processing. NIH 3T3 cells that overexpress
oncogenic H-Ras were treated with various
concentrations of FTI-277 (0-50 μM) or GGTI-286
(0-30 μM). The cells were lysed and the lysates
15   were electrophoresed on SDS-PAGE and immunoblotted
with either anti-Ras or anti-Rap1A antibodies as
described in Example 3. U and P designate
unprocessed and processed forms of the proteins.
Data are representative of three independent
20   experiments.

Figure 19.   Disruption of K-Ras4B processing.
NIH 3T3 cells that overexpress oncogenic K-Ras4B
were treated with FTI-277 or GGTI-286 (0-30 μM).
The cells were lysed and the lysates were
25   electrophoresed on SDS-PAGE and immunoblotted with
anti-Ras antibodies as described in Example 3. U
and P designate unprocessed and processed forms of
Ras. The data are representative of three
independent experiments.

30   Figure 20.   Inhibition of oncogenic activation
of MAP Kinase. NIH 3T3 cells that overexpress

either oncogenic H-Ras or K-Ras4B were treated
with either FTI-277 or GGTI-286 (0-30 μM). The
cells were lysed and the lysates were
electrophoresed on SDS-PAGE and immunoblotted with
5   an anti-MAP kinase antibody. P-MARK designates
hyperphosphorylated MAP kinase. The data are
representative of three independent experiments.

**Figure 21. Inhibition of FTase and GGTase I
Activity by GGTI-297.**

10   **Figure 22. Antitumor Efficacy of GGTI-286 in K-
Ras4B.**
<u>Description of Preferred Embodiments</u>
For ease of reference, the following
abbreviations may be used in the present
15   specification:
FTase: farnesyltransferase;

GGTase: geranylgeranyltransferase;

SDS-PAGE: sodium dodecyl sulfate
            polyacrilamide gel
20   electrophoresis

PBS: phosphate-buffered saline;

CAAX: tetrapeptide where C is cysteine, A is
            an aliphatic amino acid and X is an
            amino acid

25   DTT: dithiothreitol;

DOC: deoxycholate

BSA: bovine serum albumin

GGTase I: geranylgeranyl transferase I;

PAGE: polyacrylamide gel electrophoresis;

30   MAPK: mitogen activated protein kinase;

FTI: farnesyltransferase inhibitor;

GGTI: geranylgeranyltransferase inhibitor;

PMSF: phenylmethylsulfonyl fluoride.

I. **Farnesyltransferase Inhibitors of the type CβX**

The peptidomimetics of Formula (I), one of
the preferred embodiments of the invention, may be

5   made using procedures which are conventional in
the art. Preferably β is 2-phenyl-4-aminobenzoic
acid although constrained derivatives such as
tetrahydroisoquinoline-7-carboxylic acid, 2-
aminomethyl pyridine-6-carboxylic acid or other

10   heterocyclic derivatives, may also be used.
Compounds in which β is an aminomethylbenzoic acid
(particularly 3-aminomethylbenzoic acid) are
disclosed in U.S. Patent application No.
08/062,287, which is hereby incorporated herein by

15   reference. The acid component of β is
conveniently reacted with cysteine so that the
amino group of β and the cysteine carboxyl group
react to form an amido group, other reactive
substituents in the reactants being suitably

20   protected against undesired reaction. In the case
of the reduced-cysteine series of compounds, the
amino group of β is reacted with a suitably
protected cysteinal. The amino acid represented
by X, preferably Met, is then reacted through its

25   amino group with the deprotected and activated
carboxyl group of spacer compound β. Following
deprotection by removal of other protecting
groups, the compound of Formula (I) is obtained.

As an alternative, β may first be reacted

30   with the X amino acid followed by reaction with
the cysteine or cysteinal component using
conventional reaction conditions.

The invention also includes the
pharmaceutically acceptable salts of the compounds

35   of Formula (I). These may be obtained by reacting
the free base or acid with the appropriate amount

- 42 -

of inorganic or organic acid or base, e.g. an
alkali metal hydroxide or carbonate, such as
sodium hydroxide, an organic amine, e.g.
trimethylamine or the like. Acid salts include
5   the reaction products obtained with, for example,
toluene sulfonic acid, acetic acid, propionic acid
or the like as conventionally used in the art.

The compounds of the invention may be used to
inhibit p21ras farnesyltransferase in any host
10   containing the same. This includes both *in vitro*
and *in vivo* use. Because the compounds inhibit
farnesyltransferase, notably human tumor p21ras
farnesyltransferase, and consequently inhibit the
farnesylation of the oncogene protein ras, they
15   may be used in the treatment of cancer or cancer
cells. It is noted that many human cancers have
activated ras and, as typical of such cancers,
there may be mentioned colorectal carcinoma,
myeloid leukemias, exocrine pancreatic carcinoma
20   and the like.

The compounds of the invention may be used in
pharmaceutical compositions of conventional form
suitable for oral, subcutaneous, intravenous,
intraperitoneal or intramuscular administration to
25   a mammal or host. This includes, for example,
tablets or capsules, sterile solutions or
suspensions comprising one or more compounds of
the invention with a pharmaceutically acceptable
carrier and with or without other additives.
30   Typical carriers for tablet or capsule use
include, for example, lactose or corn starch. For
oral compositions, aqueous suspensions may be used
with conventional suspending agents, flavoring
agents and the like.
35   The amount of inhibitor administered to
obtain the desired inhibitory effect will vary but
can be readily determined. For human use, daily

dosages are dependent on the circumstances, e.g.
age and weight. However, daily dosages of from
0.1 to 20 mg per kg body weight may be mentioned
for purposes of illustration.

5   The various aspects of the invention are
further described by reference to the following
examples. These examples illustrate, among other
things, the preparation of the present
peptidomimetics and compounds compared therewith.

10   In the invention, the β component is, in
general, any non-peptide amino acid combination or
other hydrophobic spacer element that produces a
compound which mimics the structure and
conformation of CVIM or like tetrapeptides CA_1A_2X.
15   A variety of hydrophobic spacers have been used as
the β component according to this aspect of the
invention. This includes, for example, 3-
aminobenzoic acid, 4-aminobenzoic acid and 5-
aminopentanoic acid as well as heterocyclic
20   carboxylic acids such as tetrahydroiso-quinoline-
7-carboxylic acid, 2-aminomethyl pyridine-6-
carboxylic acid or the like as mentioned earlier,
as replacements for the β component of the Formula
(I) compounds. Thus, in a broad sense, the
25   peptidomimetics of the invention include variants
for Formula (I) where β stands for the radical of
a non-peptide aminoalkyl or amino-substituted
aliphatic or aromatic carboxylic acid or a
heterocyclic monocarboxylic acid, for example, 3-
30   aminobenzoic acid (3-ABA), 4-aminobenzoic acid (4-
ABA) or 5-aminopentanoic acid (5-APA).
           Other suitable β substituents which may be
mentioned include those obtained by using
aminomethyl- or aminocarboxylic acid derivatives
35   of other cyclic hydrophobic compounds such as
furan, quinoline, pyrrole, oxazole, imidazole,

pyridine and thiazole. Generally speaking,
therefore, the β substituent may be derived from
any hydrophobic, non-peptidic aminoalkyl- or
amino-substituted aliphatic, aromatic or

5   heterocyclic monocarboxylic acid.

According to still another feature of this
embodiment of the invention, other effective
inhibitors for farnesyltransferase may be provided
by incorporating a negatively charged residue onto

10   the compounds of Formula (I). This feature of the
invention is based on a consideration of the
transition state of the farnesylation reaction and
the recognition that the functional enzyme complex
must involve a farnesyl pyrophosphate binding site

15   close to the peptide binding region. Compounds
representative of this embodiment include peptides
prepared with a phosphonate residue linked at
different distances to the cysteine sulfur. These
derivatives have been prepared by reaction of N-

20   Cbz-cysteine with ethyl 2-chloroethylphosphonate
followed by condensation with the C-terminal
methionine adduct of 4-aminobenzoic acid (or N-
deprotected VIM methyl ester). Deprotection of
the phosphonate, carboxylate and amino protecting

25   groups gives analogs (5) and (6), respectively,
which contain elements of the tetrapeptide and
farnesyl pyrophosphate residues and hence are able
to interact with binding groups in both
recognition sites in p21ras farnesyltransferase:

30   (5)

(6)
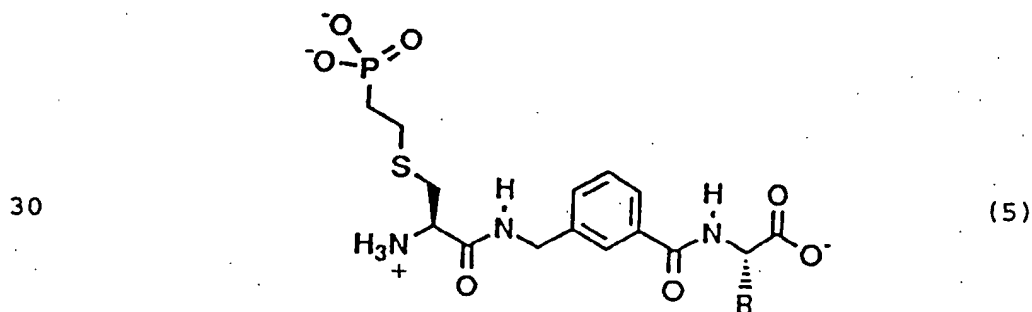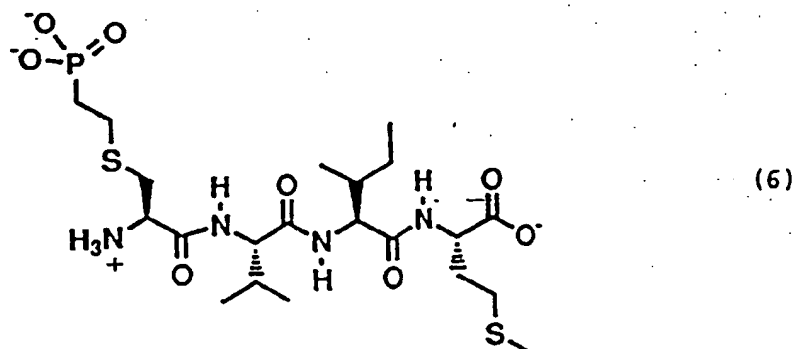
The above described phosphonates as
contemplated herein can be structurally
represented as follows:

5   Δ₁-C-β-X

where C, X, β and Δ are as previously described
and Δ₁ is a phosphonate group joined to cysteine
through the cysteine sulphur atom.

As indicated earlier, an important further
10   feature of the invention is the modification of
the compounds of the invention, as well as the
tetrapeptide p21ras farnesyl transferase
inhibitors of the formula CA₁A₂X, to provide pro-
drugs. This involves forming lipophilic enzyme-
15   sensitive derivatives from the compounds by
appropriately functionalizing the terminal groups.
For example, the terminal amino groups and the
cysteine sulfur can be reacted with benzyl
chloroformate to provide carbobenzyloxy ester end
20   groups while the terminal carboxy group at the
other end of the compound is converted to an alkyl
or aryl ester, e.g. the methyl ester.   Other

- 46 -

examples include alkyl esters from 1 to 10 carbons
in length, activated esters such as cyanomethyl or
trifluoromethyl, cholesterol, cholate or
carbohydrate derivatives. The term "lipophilic",
5   when used in this context, is meant to include,
inter alia, methoxycarbonyl and other long chain
or carbamate groups. Examples of such groups are
well known to the ordinarily skilled practitioner.

Derivatization of the prior peptides CA_1A_2X
10   and the peptidomimetics described herein with
lipophilic or hydrophobic, enzyme-sensitive
moieties increases the plasma membrane
permeability and cellular uptake of the compounds
and consequently their efficiency in inhibiting
15   tumor cell growth.

While the carbobenzyloxy derivatives have
been referred to as one way of functionalizing the
peptides and peptidomimetics to improve
efficiency, it will be appreciated that a variety
20   of other groups may also be used for the purposes
noted. Typical alternatives include
cholesterolyl, aryl or aralkyl such as benzyl,
phenylethyl, phenylpropyl or naphthyl, or alkyl,
typically methyl or other alkyl of, for example,
25   up to 8 carbon atoms or more. It is contemplated
that such functional groups would be attached to
the cysteine sulfur and the terminal amino and
carboxy groups.

Using C-ABA-M as representative of the
present compounds, the functionalized pro-drug
embodiment of the invention may be structurally
illustrated as follows:

5

BBM-CABAM
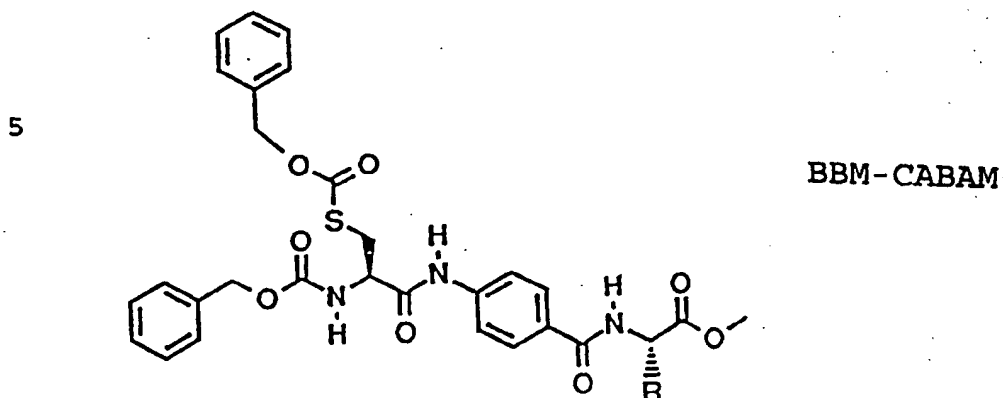
In the above described BBM-compounds, the
"BBM" used in the formulas represents a shorthand
reference to the bis-(carboxybenzyloxy)methyl
10   esters of CβM and CVIM.

The functionalized derivatives of the
phosphonates described earlier herein are also
useful cell growth inhibitors. Correspondingly,
the "BMMM" designation used with compounds refers
15   to the carboxy benzyloxy substitution and the
three methyl groups in the methylated phosphoric
and carboxylic acid end groups.

As noted, the purpose of the functional
groups added to the parent compounds is to improve
20   entry of the compounds into tumor cells. Once in
the cells, the functional groups are removed to
liberate the active compound to function in its
inhibitory capacity.

As will be recognized by those in the art,
25   the functionalized pro-drugs of the invention can
be prepared using conventional and well-known
procedures for esterifying amino, SH and

- 48 -

carboxylic acid groups. Hence, details of such
procedures are not essential for the preparation
of the present pro-drugs.

EXAMPLE 1

5   <u>SYNTHESIS OF FTI-232</u>

A.   N-BOC-4-aminobenzoic acid

4-amino-benzoic acid (10g, 72.9 mmol) was
placed into a mixture of dioxane (145.8 ml) and
0.5M NaOH (145.8 ml). The solution was cooled to

10   0°C and di-t-butyl dicarbonate (23.87 g, 109.5
mmol) was added. The reaction mixture was allowed
to warm to room temperature and stirred overnight.
The next day, the dioxane was removed, the residue
was made acidic and extracted into ethyl acetate.

15   The ethyl acetate fractions were combined and
washed with 1N HCl to remove any unreacted
starting material. The solution was dried over
Na_2SO_4 and the solvent was removed in vacuo. The
crude material was recrystallized from ethyl

20   acetate/hexanes to yield 12.2 g (70.6%) of pure
product. mp 189-190°C; ^1H NMR (CD_3OD) 1.52 (9H,
s), 7.49 (2H, d, J=8.6 Hz), 7.91 (2H, d, J=8.6
Hz), 9.28 (1H, s); ^{13}C NMR (CD_3OD) 28.59, 81.29,
118.54, 125.30, 131.81, 145.70, 155.00, 169.80;

25   anal. calc. for $C_{12}H_{15}NO_4$, C: 60.76, H: 6.37, N:
5.90; found, C: 60.52, H: 6.43, N: 5.83; HRMS
calc. for $C_{12}H_{15}NO_4$, 237.0961, found, 237.1001.

B.   N-BOC-4-aminobenzoyl methionine methyl ester

Into a dried, nitrogen filled flask was

30   placed N-BOC-4-aminobenzoic acid (8.77 g, 36.97
mmol) in dry CH_2Cl_2 (148 ml) along with methionine
methyl ester hydrochloride (8.12 g, 40.66 mmol).
This solution was cooled in an ice bath and
triethylamine (6.7 ml), EDCI (7.80 g, 40.66 mmol)

35   and hydroxybenzotriazole (HOBT, 5.50 g, 40.66

mmol) were added. The mixture was stirred
overnight, diluted with more CH_2Cl_2 and was
extracted 3 times each with 1M HCl, 1M $NaHCO_3$ and
water. The CH_2Cl_2 was dried over $MgSO_4$ and the
solvent was removed in vacuo. The solid was
recrystallized from ethyl acetate/ hexanes to
yield 9.72 g (71.3%) of pure product. mp 184-
185°C; ^1H NMR $(CDCl_3)$ 1.53 (9H, s), 2.06-2.18 (4H,
m), 2.23-2.33 (1H, m), 2.59 (2H, t, J=7.6 Hz),
3.80 (3H, s), 4.92 (1H, m), 7.45 (2H, d, J=8.7
Hz), 7.77 (2H, d, J=8.7 Hz); ^{13}C NMR $(CDCl_3)$ 15.59,
28.34, 30.15, 31.64, 52.10, 52.73, 81.20, 117.73,
127.8, 128.33, 141.88, 152.33, 166.50, 172.75;
anal. calc. for $C_{18}H_{26}N_2O_5S$, C: 56.53, H: 6.85, N:
7.29; found, C: 56.47, H: 6.86, N: 7.29; m/ z (EI)
382 (M).

C. HCl-4-aminobenzoyl methionine methyl ester

N-BOC-4-aminobenzoyl methionine methyl ester
(3.53 g, 9.59 mmol) was placed into CH_2Cl_2 (30-35
ml) and to it was added 3M HCl/ Et_2O (38.4 ml).
After standing a white precipitate formed. After
2 hours the solution was decanted, and the
crystals were collected by centrifugation. The
crystals were then washed several times with fresh
ether and dried overnight on the vacuum pump.
Meanwhile, the filtrate was left to stand
overnight to allow additional product to
precipitate. The second fraction was washed with
ether and dried overnight on the vacuum pump. The
total yield of pure fully deprotected material was
2.87 g (93.9%) yield. mp 158-164°C; ^1H NMR $(CDCl_3)$
2.10 (3H, s), 2.12-2.29 (1H, m), 2.52-2.71 (1H,
m), 2.59 (2H, t, J=7.6 Hz), 3.75 (3H, s), 4.79
(1H, m), 7.02 (2H, d, J=8.6 Hz), 7.55 (2H, d,
J=8.6 Hz); ^{13}C NMR $(CDCl_3)$ 15.23, 31.43, 31.53,
52.91, 52.43, 124.35, 130.56, 135.31, 135.76,

168.95, 173.87; HRMS calc. for $C_{13}H_{18}N_2O_3S$, 282.1038, found 282.1009.

D. N-BOC-S-trityl-cysteine-4-aminobenzoyl methionine methyl ester

5   N-BOC-S-trityl-Cys (2.86 g, 6.54 mmol) and triethylamine (1.2 ml) were placed into a dried, N_2 filled flask containing dry THF (104 ml). This was cooled to -10°C using an ice/ salt bath and isobutyl chloroformate (0.9 ml), IBCF, was added.

10   The solution was stirred at -10°C for 40 minutes and HCl-4-aminobenzoyl methionine methyl ester (2.08 g, 6.54 mmol) in dry CH_2Cl_2 (34.1 ml) with triethylamine (1.2 ml, 1.3 eq) was added. The solution warmed to room temperature and was

15   stirred overnight under N_2. The solvent was then removed in vacuo and the residue was taken up in CH_2Cl_2 and extracted several times each with 1M HCl, H_2O and brine (saturated NaCl). The organic layer was dried over Na_2SO_4 and the solvent was

20   removed in vacuo. The pale yellow foam was then chromatographed on silica gel using a 2:1 hexanes, ethyl acetate elution mixture to yield 2.62 g (54.9%) of pure product. mp 110-111°C; $[\alpha]^{25}_D = -8.0°$ (c=1, CH_3OH); ^1H NMR ($CDCl_3$) 1.44 (9H,s),

25   2.11-2.18 (4H, m), 2.22-2.34 (1H,m), 2.59 (2H, t, J=7.4 Hz), 2.66-2.83 (2H, m), 3.80 (3H, s), 3.98 (1H, m), 4.84 (1H, m), 4.92 (1H, m), 6.96 (1H, d, J=7.7 Hz), 7.23-7.33 (9H, m), 7.43-7.46 (6H, m), 7.51 (2H, d, J=8.5 Hz), 7.74 (2H, d, J=8.5 Hz),

30   8.51 (1H, s); ^{13}C NMR ($CDCl_3$) 15.53, 28.34, 30.72, 30.89, 33.60, 52.23, 52.88, 54.95, 60.50, 67.13, 80.64, 118.81, 119.31, 126.94, 128.07, 128.30, 129.53, 141.06, 144.38, 156.31, 167.02, 170.13, 174.49; anal calc for $C_{40}H_{44}N_3O_6S_2 \cdot H_2O$, C: 64.50, H:

35   6.22, N: 5.64; found C: 64.14 H: 6.19, N: 5.56.

E.   HCl-cysteine-4-aminobenzoyl methionine methyl ester

N-BOC-S-trityl-cysteine-4-aminobenzoyl
methionine methyl ester   (1 g, 1.37 mmol) was

5   placed into a flask and taken up in CH_3OH (13.7
ml).   To this solution was added a solution of
mercuric chloride (0.75 g, 2.74 mmol) in CH_3OH
(13.7 ml).   Upon addition of the mercuric
chloride, a white precipitate began to form.   The

10   mixture was heated on a steam bath at 65°C for 35
minutes and then it was cooled and the precipitate
was filtered and washed sparingly with cold CH_3OH.
After drying for several minutes on the filter,
the solid was placed into a 50 ml 3-neck flask

15   fitted with a gas inlet and outlet.   Approximately
20-30 ml of CH_3OH was added and H_2S gas was bubbled
through the heterogeneous solution for 30 minutes.
Upon addition of the gas, the white solution
turned orange and then black.   The solution was

20   centrifuged and the clear, colorless liquid was
dried to give a white foam.   This solid was placed
on the vacuum pump for a short period and then was
taken up in CH_2Cl_2 (10 ml) and the product was
precipitated with a 3-4M HCl/ Et_2O solution.   The

25   precipitate was collected by centrifugation and
was washed with ether until pH was neutral.   After
drying under vacuum overnight, 0.38 g (66.5%) of
product was obtained that was >95% pure by HPLC.
mp foamed 141-143°C, decomp 195°C; $[\alpha]^{25}_D$=+3° (c=1,

30   H_2O); ^1H NMR (CD_3OD) 2.09 (3H, s), 2.14-2.26
(1H,m), 2.51-2.67 (3H, m), 3.05 (1H, dd, J=14.8
Hz, 7.3 Hz), 3.17 (1H, dd, J=14.8 Hz, 4.8 Hz),
3.74 (3H,s), 4.17 (1H, J=7.3 Hz, 4.8 Hz), 4.75-
4.81 (1H, m), 7.74 (2H, d, J=8.6 Hz), 7.87 (2H, d,

35   J=8.6 Hz), 8.67 (1H, d, J=8.4 Hz); ^{13}C NMR (CD_3OD)
15.23, 26.38, 31.43, 31.56, 52.88, 53.30, 56.92,
120.46, 129.58, 130.75, 142.33, 166.91, 169.66,

174.06; anal calc for $C_{16}H_{24}ClN_3O_4S_2$, C: 45.55 H:
5.73, N: 9.96; found C: 45.31, H: 5.84, N: 9.79.

F.   HCl-cysteine-4-aminobenzoyl methionine FTI-232

5
HCl-cysteine-4-aminobenzoyl methionine methyl
ester (0.51 g, 0.7 mmol) was taken up in THF (4.1
ml) and to this solution was added 0.5 M LiOH (2.9
ml) at 0°C. The heterogeneous solution was
stirred at 0°C for 35-40 minutes and then the THF
was removed in vacuo. The residue was taken up in

10
CH_2Cl_2 and was washed three times with 1M HCl
followed by brine. The organic solution was dried
over Na_2SO_4 and the solvent was removed in vacuo.
The pale yellow solid was taken up in 3 ml of
CH_2Cl_2 and the product was precipitated with 3-4 M

15
HCl/ Et_2O. The solid was collected by
centrifugation, washed several times with ether
until the ether washings were neutral and the
process repeated until the HPLC appeared pure. A
final yield of 78.6 mg (27.5%) of pure product was

20
obtained. mp sub 157°C, decomp 211°C; $[\alpha]^{25}_D=+10°$.
(c=0.8, H_2O); ^1H NMR (CD_3OD) 2.09 (3H, s), 2.17-
2.32 (1H,m), 2.53-2.66 (3H, m), 3.06 (1H, dd,
J=14.6 Hz, 7.2 Hz), 3.19 (1H, dd, J=14.6 Hz, 4.6
Hz), 4.21 (1H, dd, J=7.23 Hz, 4.63 Hz), 4.73-4.78

25
(1H, m), 7.75 (2H, d, J=8.1 Hz), 7.87 (2H, d,
J=8.1 Hz); ^{13}C NMR (CD_3OD) 15.23, 26.33, 31.58,
31.86,   53.24, 56.98, 120.48, 129.59, 131.10,
142.26, 166.89, 169.66, 175.29; anal calc for
$C_{15}H_{22}ClN_3O_4S_2$, C: 44.16, H: 5.44, N: 10.30; found C:

30
45.45, H: 5.62, N: 10.03; m/ z (FAB) for free
amine, 371 (M+ 1).

EXAMPLE 2

SYNTHESIS OF FTI-260

A. N-BOC-4-amino-3-methylbenzoic acid

5

10

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4-amino-3-methylbenzoic acid (5 g, 33.1 mmol)
was reacted according to the same procedure as N-
BOC-4-aminobenzoic acid. The orange-brown solid
was recrystallized from ethyl acetate and hexanes
to yield 4.99 g (60%) of tan prismatic crystals.
mp 180-182°C; ^{1}H NMR (CD$_3$OD) 1.51 (9H, s), 2.27
(3H, s), 7.66 (1H, d, J=8.1 Hz), 7.79-7.82 (2H,
m), 8.32 (1H, s); ^{13}C NMR (CD$_3$OD) 17.98, 28.62,
81.47, 123.12, 127.05, 129.14, 130.65, 132.99,
142.45, 155.33, 168.70; anal calc for C$_{13}$H$_{17}$NO$_4$, C:
62.15, H: 6.82, N: 5.58; found C: 62.07, H: 6.86,
N: 5.46; m/ z (EI) 251; HRMS calc. for C$_{13}$H$_{17}$NO$_4$,
251.1158; found, 251.1153.

**B. N-BOC-4-amino-3-methylbenzoyl methionine
methyl ester**

20

25

30

35

N-BOC-4-amino-3-methylbenzoic acid (2.00 g,
7.96 mmol) was reacted with methionine methyl
ester hydrochloride (1.75 g, 8.76 mmol), EDCI
(1.68 g, 8.76 mmol), HOBT (1.18 g, 8.76 mmol) and
Et$_3$N (1.4 ml) in dry CH$_2$Cl$_2$ (31.8 ml) according to
the procedure described for N-BOC-4-aminobenzoyl
methionine methyl ester in Example 1. The crude
material was recrystallized from ethyl acetate and
hexanes to yield 2.61 g (85.7%) of pure product.
mp 163-165°C; ^{1}H NMR (CDCl$_3$) 1.54 (9H,s), 2.06-2.18
(4H, m), 2.23-2.34 (4H, m), 2.59 (2H, t, J=6.8
Hz), 3.80 (3H, s), 4.92 (1H, m), 6.45 (1H, s),
6.88 (1H, d, J=7.5 Hz), 7.63 (1H, d, J=8.6 Hz),
7.66 (1H, s), 8.05 (1H, d, J=8.6); ^{13}C NMR (CDCl$_3$)
15.47, 17.61, 28.22, 30.03, 31.55, 51.93, 52.57,
81.04, 118.73, 125.62, 127.66, 129.54, 139.89,
152.34, 166.58, 172.66.

C. HCl-4-amino-3-methylbenzoyl methionine methyl ester

N-BOC-4-amino-3-methylbenzoyl methionine
methyl ester (0.99 g, 2.59 mmol) was dissolved in
5   CH_2Cl_2 (15-20 ml) and precipitated with 3M HCl/
Et_2O (20.7 ml). 0.83 g (96.6%) of pale orange
precipitate was obtained after drying overnight on
the vacuum pump. mp 157-159°C; ^{1}H NMR (CD_3OD) 2.04
(3H,s), 2.11-2.25 (1H, m), 2.47 (3H, s), 2.52-2.68
10   (3H. m), 3.74 (3H, s), 4.75-4.80 (1H, m), 7.48
(1H, d, J=8.2 Hz), 7.81 (2H, d, J=8.2 Hz), 7.87
(1H, s); ^{13}C NMR (CD_3OD) 15.23, 17.28, 31.43,
31.51, 52.91, 53.37, 124.41, 127.85, 131.99,
133.63, 134.14, 135.65, 169.05, 173.84; anal.
15   calc. for $C_{14}H_{21}N_2O_3S$, C: 50.52, H: 6.36, N: 8.42;
found C: 50.71, H: 6.40, N: 8.34.

D. N-BOC-S-trityl-cysteine-4-amino-3-methylbenzoyl methionine methyl ester

N-BOC-S-trityl-cysteine (0.55 g, 1.25 mmol)
20   in dry THF (25 ml) was reacted with Et_3N (0.19 ml),
IBCF (0.16 ml, 1.25 mmol) at -10 °C as described
above. HCl-4-amino-3-methylbenzoyl methionine
methyl ester (0.42 g, 1.25 mmol) in dry CH_2Cl_2
(6.5 ml) with Et_3N (0.26 ml) was added at -10°C and
25   the reaction mixture was allowed to stir overnight
under nitrogen. Workup was carried out as
described above and the crude material was
chromatographed on silica gel using a 2:1 mixture
of hexanes and ethyl acetate as an elution mixture
30   to give 0.12 g (13.9%) of pure product. mp 83-
85°C; $[\alpha]^{25}_D$=-14.0° (c=1, CH_3OH); ^{1}H NMR ($CDCl_3$) 1.44
(9H,s), 2.10-2.17 (4H, m), 2.22-2.32 (4H, m), 2.61
(2H, t, J=6.57 Hz), 2.68-2.70 (1H, m), 2.85-2.90
(1H. m), 3.79 (3H,s), 3.93-4.08 (1H, s), 4.84-4.88
35   (1H, m), 4.90-4.95 (1H, m), 6.95 (1H, d, J=7.00
Hz), 7.20-7.33 (9H,m), 7.39 (1H, d, J=6.96 Hz),

7.44-7.47 (6H,m), 7.59 (1H, d, J=8.46 Hz), 7.65 (
1H, s), 8.12 (1H,d, J=8.22 Hz), 8.31 (1H,s); ^{13}C
NMR (CDCl$_3$) 15.39 17.55, 27.70, 28.17, 30.00,
31.43, 31.41, 51.90, 52.51, 59.95, 67.30. 80.74,
5 84.54, 120.74, 125.33, 126.70, 126.83, 127.89,
128.00, 129.40, 138.92, 144.22, 166.50, 166.89,
168.87, 172.56.

E. TFA·cysteine-4-amino-3-methylbenzoyl methionine FTI-260

10 N-BOC-S-trityl-cysteine-4-amino-3-
methylbenzoyl methionine methyl ester (0.27 g,
0.37 mmol) in THF (2.1 ml) was deprotected with
0.5M LiOH (2.9 ml) over 1.5 h at room temperature.
The solvent was removed in vacuo and the residue
15 was taken up in CH$_2$Cl$_2$ and extracted 3 times with
1N HCl followed by extraction with brine. The
organic solution was dried over Na$_2$SO$_4$ and the
solvent was removed in vacuo to give 0.19 g (73.5
%) of the free acid. The free acid was then taken
20 up in CH$_2$Cl$_2$ (1.4 ml) and Et$_3$SiH (0.04 ml) was added
followed by trifluoroacetic acid, TFA (1.4 ml).
The reaction mixture was stirred at room
temperature for 1 hour. The TFA was removed and
the residue was dissolved in H$_2$O and extracted with
25 Et$_2$O until all of the trityl derivative was
removed. The water was lyophilized and a crude
HPLC showed that the material was impure and
contained diastereomers. The product was purified
on the preparative HPLC using 0.1% TFA in water
30 and acetonitrile elution mixture to give 2
diastereomers and only the major component
(determined according to the major compound in the
HPLC trace) was characterized. mp sub 112°C,
foamed 158-163°C, decomp 196-197°C; $[\alpha]^{25}_D$=+12.7°
35 (c=0.6 H$_2$O), $[\alpha]^{25}_D$=+ 21.0° (c=1 H$_2$O); ^{1}H NMR (CD$_3$OD)
2.09-2.17 (4H, m), 2.19-2.30 (1H, m), 2.36 (3H,

s), 2.57-2.65 (2H, m), 3.08 (1H, dd, J=14.6 Hz,
6.9 Hz), 3.19 (1H, dd, J=14.6 Hz, 5.2 Hz), 4.25 (
1H, dd, J=6.9, 5.2 Hz), 4.70-4.75 (1H, m), 7.64
(1H, d, J=8.4 Hz), 7.69-7.73 (1H, m), 7.77 (1H,

5   s); ^{13}C NMR (CD$_3$OD) 15.23, 18.28, 26.54, 31.58,
32.06, 53.53, 56.66, 125.54, 125.77, 126.74,
131.04, 133.24, 139.26, 167.53, 169.70, 175.59.

EXAMPLE 3

SYNTHESIS OF FTI-261

10   **A.   N-BOC-4-amino-3-methoxybenzoic acid**

4-amino-3-methoxybenzoic acid (1 g, 5.98
mmol) was reacted with di-t-butyl dicarbonate
(1.96 g, 6.58 mmol) in dioxane (12 ml) and 0.5 M
NaOH (12 ml) according to the same procedure as N-

15   BOC-4-aminobenzoic acid. 1.50 g (93.7%) of tan
crystals were obtained after recrystallization
from ethyl acetate and hexanes. mp 176-178°C; ^{1}H
NMR (CD$_3$OD) 1.52 (9H, s), 3.92 (3H, s), 7.56 (1H,
s), 7.62 (1H, d, J=8.4 Hz), 7.96 (1H, s), 8.03

20   (1H, d, J=8.4 Hz); ^{13}C NMR (CD$_3$OD) 28.53, 56.35,
81.78, 112.01, 118.58, 124.20, 125.76, 133.84,
149.04, 154.20, 169.60; HRMS calc. for C$_{13}$H$_{17}$NO$_5$,
267.1107; found, 267.1103.

B.   N-BOC-4-amino-3-methoxybenzoyl methionine
25   **methyl ester**

N-BOC-4-amino-3-methoxybenzoic acid (0.35 g,
1.31 mmol) was reacted with methionine methyl
ester hydrochloride (0.9 g, 1.43 mmol) using EDCI
as in N-BOC-4-aminobenzoyl methionine methyl

30   ester. After recrystallization from ethyl acetate
and hexanes, 0.36 g (57.2 %) of pure product was
obtained. mp 163-165°C; ^{1}H NMR (CDCl$_3$) 1.53 (9H,
s), 2.09-2.18 (4H, m), 2.23-2.35 (1H, m), 2.60
(2H, t, J=6.9 Hz), 3.80 (3H, s), 3.93 (3H, s),

35   4.92 (1H, br s), 6.93 (1H, d, J=7.6 Hz), 7.25 (1H,

m), 7.31 (1H, d, J=10.2 Hz), 7.44(1H, s), 8.15(1H,
d, J=8.5 Hz); ^{13}C NMR (CDCl$_3$) 15.47, 28.23, 30.09,
31.48, 52.06, 52.54, 55.81, 80.82, 98.06, 109.38,
116.66, 119.31, 131.52, 147.23, 152.31, 166.57,
5   172.58; m/ z (FAB) 413 (M+1).

C. HCl-4-amino-3-methoxybenzoyl methionine methyl
ester

       N-BOC-4-amino-3-methoxybenzoyl methionine
methyl ester (0.71 g, 1.79 mmol) was taken up in
10   CH$_2$Cl$_2$ (4 ml) and precipitated with 3-4M HCl/ Et$_2$O
(12 ml). The precipitate was washed as usual with
Et$_2$O and dried overnight under vacuum to result in
0.55 g (88.3%) of reddish material. mp 176-177°C;
^{1}H NMR (CD$_3$OD) 2.08 (3H, s), 2.21 (2H, m), 2.61
15   (2H, m), 3.74 (3H, s), 4.02 (3H, s), 4.79 (1H, m),
7.50 (1H, d, J=8.2 Hz), 7.57 (1H, d, J=4.1 Hz)
7.67 (1H, s); ^{13}C NMR (CD$_3$OD) 15.26, 31.34, 31.42,
52.95, 53.38, 57.12, 112.29, 121.43, 124.57,
124.77, 136.15, 153.67, 168.79, 173.81.

20   D. N-BOC-S-trityl-cysteine-4-amino-3-
methoxybenzoyl methionine methyl ester
       N-BOC-S-trityl-cysteine (0.76 g, 1.74 mmol)
in dry THF (27.5 ml) was reacted with Et$_3$N (0.24
ml), IBCF (0.23 ml, 1.74 mmol) at -10°C as
25   described above. HCl-4-amino-3-methoxybenzoyl
methionine methyl ester (0.55 g, 1.58 mmol) in
dry CH$_2$Cl$_2$ (8.7 ml) with Et$_3$N (0.30 ml) was added
to the mixture and was allowed to stir overnight
under nitrogen. It was worked up as described for
30   N-BOC-S-trityl-cysteine-4-aminobenzoyl methionine
methyl ester in Example 1, and the crude material
was chromatographed on silica gel using a 2:1
mixture of hexanes and ethyl acetate to give 0.18
g (15.2%) of pure product. ^{1}H NMR (CDCl$_3$) 1.45
35   (9H, s), 2.05-2.33 (5H, m), 2.57-2.65 (2H, m),

- 58 -

2.68-2.72 (1H, m), 2.75-2.96 (1H, m), 3.78 (3H,
s), 3.84 (3H, s), 4.90-5.00 (1H, m), 5.03-5.18
(1H, m), 7.17-7.48 (17H, m), 8.30-8.38 (1H, m),
8.65 (1H, br s).

5   **E. TFA·Cysteine-4-amino-3-methoxybenzoyl
methionine FTI-261**
         N-BOC-S-trityl-cysteine-4-amino-3-
methoxybenzoyl methionine methyl ester   (0.18 g,
0.24 mmol) was deprotected with LiOH at room
10   temperature as described above to give the free
acid. The free acid was then further deprotected
in CH_2Cl_2 (1.2 ml) with Et_3SiH (0.04 ml, 0.24 mmol)
and TFA (1.2 ml). The product was worked up as
described for HCl-cysteine-4-aminobenzoyl
15   methionine in Example 1, and HPLC revealed that
the product was impure. The crude material was
then purified on the HPLC using 0.1% TFA in water
and acetonitrile as eluting solvents to result in
two pure samples that were expected to be
20   diastereomers. The major component (determined
according to the major compound in the HPLC trace)
was characterized as follows. mp sub 109°C,
decomp 191-193°C; $[\alpha]^{25}_D=-30.0°$ (c=1, H_2O),
$[\alpha]^{25}_D=+19.0°$ (c=1, H_2O); ^1H NMR (CD_3OD) 2.10 (3H,
25   s), 2.12-2.18 (1H, m), 2.20-2.32 (1H, m), 2.53-
2.71 (2H, m), 3.00 (1H, dd, J=14.6, 7.5), 3.15
(1H, dd, J=14.58, 4.8), 4.77 (1H, dd, J=7.5, 4.8),
7.50 (1H, d, J=8.4 Hz), 7.56 (1H, s), 8.23 (1H, d,
J=8.4 Hz); ^{13}C NMR (CD_3OD) 15.20, 26.65, 31.60,
30   31.76, 53.27, 56.58, 56.76, 111.04, 121.08,
122.14, 130.85, 131.85, 150.88, 167.21, 169.61,
175.36; m/ z (FAB) for free amine, 402 (M+1).

EXAMPLE 4

<u>SYNTHESIS OF FTI-272</u>

A.   4-nitro-2-phenyltoluene

2-bromo-4-nitrotoluene (2.16 g, 10.00 mmol)

5   and phenyl boric acid (1.46 g, 12.00 mmol) were
dissolved into anhydrous DMF (25 ml) under
nitrogen. To this mixture was added $Pd(Ph_3P)_4$
(0.58 g, 5%). The mixture was heated at 100 °C
overnight. The solution was poured onto 1N HCl

10   and extracted with Et_2O. The crude material was
chromatographed on silica gel using hexanes as an
eluent. After recrystallization from ethanol,
1.23 g (57.6%) of pale orange needles were
obtained. mp 69 - 71°C; ^1H NMR ($CDCl_3$) 2.36 (3H,

15   s), 7.29-7.40 (2H, m), 7.41-7.49 (5H,m), 8.07-8.10
(2H, m); ^{13}C NMR ($CDCl_3$) 20.68, 121.96, 124.51,
127.78, 128.41, 128.83, 131.06, 139.44, 142.97,
143.48, 146.05; anal calc. for $C_{13}H_{11}NO_2$, C:73.26,
H:5.20, N:6.57; found, C:73.10, H:5.12, N:6.50; m/

20   z (EI) 213; HRMS calc. for $C_{13}H_{11}NO_2$, 213.0790;
found, 213.0793.

B.   4-nitro-2-phenylbenzoic acid

4-nitro-2-phenyltoluene (0.50 g, 2.34 mmol)
was dissolved in water (4.6 ml) and pyridine (2.3

25   ml). The mixture was heated to reflux and $KMnO_4$
(1.85 g, 11.70 mmol) was added. The reaction
mixture was heated overnight and the solution was
filtered and washed several times with boiling
water. The aqueous solution was made acidic and

30   the product was extracted into ethyl acetate. The
ethyl acetate was dried over Na_2SO_4 and the solvent
removed in vacuo to result in 0.37 g (67.9%) of
pure yellow product. mp 174-176°C; ^1H NMR (CD_3OD)
7.38-7.48 (5H, m), 7.96 (1H, d, J=8.5 Hz), 8.21

35   (1H, d, J=2.3 Hz), 8.28 (1H, dd, J=8.48, 2.37); ^{13}C
NMR (CD_3OD) 122.95, 126.09, 129.27, 129.42, 129.49,

131.56, 139.26, 140.42, 144.41, 150.17, 170.52; m/
z (EI) 243 (M).

C.   4-nitro-2-phenylbenzoyl methionine methyl ester

5   4-nitro-2-phenylbenzoic acid (0.30 g, 1.23
mmol), methionine methyl ester hydrochloride salt
(0.27 g, 1.35 mmol), EDCI (0.26 g, 1.35 mmol),
HOBT (0.18 g, 1.35 mmol) and Et_3N (0.19 ml) in dry
CH_2Cl_2 (4.9 ml) were reacted according to the above
10   procedure and worked up as described for N-BOC-4-
aminobenzoyl methionine methyl ester in Example 1.
After recrystallization from ethyl acetate and
hexanes, 0.41 g (85.5%) of pure product was
isolated.   mp 98-101°C; ^1H NMR ($CDCl_3$) 1.62-1.73
15   (1H, m), 1.79-1.88 (1H, m), 1.91 (3H, s), 1.99
(2H, t, J=7.2 Hz), 3.59 (3H, s), 4.53 (1H, m),
6.45 (1H, d, J=7.8 Hz), 7.33-7.40 (5H, m), 7.67
(1H, d, J=8.3 Hz), 8.07-8.12 (2H, m); ^{13}C NMR
($CDCl_3$) 14.92, 29.11, 30.67, 51.51, 52.29, 121.86,
20   124.74, 128.27, 128.60, 128.69, 129.52, 137.50,
140.56, 141.02, 148.09, 167.23, 171.23; m/ z
(FAB), 389 (M+1).

D.   4-amino-2-phenylbenzoyl methionine methyl ester

25   4-nitro-2-phenylbenzoyl methionine methyl
ester (0.35 g, 0.90 mmol) was taken up in ethyl
acetate (9.0 ml). To this mixture was added
$SnCl_2 \cdot 2H_2O$ (1.02 g, 4.50 mmol) and the reaction was
heated under nitrogen at reflux for 1h. The
30   mixture was poured onto ice, the solution was made
basic using $NaHCO_3$ and the product was extracted
into ethyl acetate several times (7-8). The ethyl
acetate fractions were combined washed with brine
and dried over Na_2SO_4 and the solvent was removed
35   in vacuo to give 0.24 g (73.4%) of yellow solid.

^{1}H NMR (CDCl$_3$) 1.58-1.70 (1H, m), 1.80-1.92 (1H, m), 1.98 (3H, s), 2.06 (2H, t, J=7.7 Hz), 3.62 (3H, s), 4.00 (2H, br s), 4.56-4.63 (1H, m), 5.84 (1H, d, J=7.7 Hz), 6.50 (1H, s), 6.61 (1H, d, J=8.4 Hz), 7.29-7.42 (5H, m), 7.58 (1H, d, J=8.3 Hz); ^{13}C NMR (CDCl$_3$) 15.02, 29.25, 31.25, 51.57, 52.15, 113.27, 115.88, 123.52, 127.56, 128.37, 128.44, 130.92, 140.66, 141.44, 148.53, 168.58, 171.91.

E. N-BOC-S-trityl-cysteine-4-amino-2-phenylbenzoyl methionine methyl ester

N-BOC-S-trityl-cysteine (0.31 g, 0.66 mmol) in dry THF (11 ml) was reacted with Et$_3$N (0.10 ml), IBCF (0.09 ml, 0.73 mmol) at -10 °C as described for N-BOC-S-trityl-cysteine-4-aminobenzoyl methionine methyl ester in Example 1. 4-amino-2-phenylbenzoyl methionine methyl ester (0.24g, 0.66 mmol) in dry CH$_2$Cl$_2$ (3.5 ml) was added and the mixture was allowed to stir overnight under nitrogen. It was worked up as described as further described for N-BOC-S-trityl-cysteine-4-aminobenzoyl methionine methyl ester in Example 1, and after drying the crude material was chromatographed on silica gel using a 2:1 mixture of hexanes and ethyl acetate to give 84.70 mg (16.0%) of pure product. mp 100-103°C; ^{1}H NMR (CDCl$_3$) 1.41 (9H,s), 1.61-1.78 (1H, m), 1.84-1.95 (1H, m), 2.00 (3H, s), 2.05 (2H, t, J=7.6 Hz), 2.63 (1H, dd, J=12.7 Hz, 6.9 Hz), 2.72 (1H, dd, J=12.7 Hz, 5.51 Hz), 3.64 (3H, s), 4.02 (1H, br s), 4.58-4.63 (1H, m), 4.90 (1H, d, J=7.4 Hz), 6.10 (1H, d, J=6.6 Hz), 7.18-7.30 (10H, m), 7.37-7.44 (11H, m), 7.50 (1H, s), 7.58 (1H, d, J=8.2 Hz), 8.69 (1H, s); ^{13}C NMR (CDCl$_3$) 15.21, 28.20, 29.38, 31.24, 33.00, 51.77, 52.35, 54.15, 67.30, 80.85, 118.18, 120.86, 126.88, 127.90, 128.03,

128.56, 128.66, 129.44, 129.79, 130.14, 156.00,
168.52, 169.11, 171.85.

F.   TFA·Cysteine-4-amino-2-phenylbenzoyl
methionine FTI-272

N-BOC-S-trityl-cysteine-4-amino-2-
phenylbenzoyl methionine methyl ester   (84.70 mg,
0.11 mmol) of was taken up in THF (0.62 ml) and to
this was added 0.5 M LiOH (0.32 ml) at 0 °C. The
mixture was stirred at 0 °C for 35 minutes. The
solvent was removed in vacuo using a cold water
bath on the rotovap. The residue was worked up as
described for HCl-cysteine-4-aminobenzoyl
methionine in Example 1, and 60 mg of the free
acid was obtained. This was then dissolved into
CH_2Cl_2 (0.8 ml) and Et_3SiH (0.01 ml) was added
followed by TFA (0.8 ml). The reaction mixture
was stirred at room temperature for 1 h and worked
up as described for TFA·cysteine-4-amino-3-
methylbenzoyl methionine in Example 2. After
lyophilization, 0.0387 g (84.0%) was obtained.
HPLC revealed that no epimerization had occurred,
however the material was purified on the HPLC to
eliminate baseline impurities. mp 150-154°C;
$[\alpha]^{25}_D$=+21.5° (c=0.7, H_2O/ CH_3OH); ^1H NMR (CD_3OD)
1.62-1.79 (1H, m), 2.00-2.10 (5H, m), 2.16-2.18
(1H, m), 3.03 (1H, dd, J=14.7 Hz, 7.3 Hz), 3.15
(1H, dd, J=14.7 Hz, 4.8 Hz), 4.46 (1H, br s),
7.37-7.41 (5H, m), 7.52- 7.55 (1H, m), 7.65-7.67
(2H, m); ^{13}C NMR (CD_3OD) 15.03, 26.35, 31.78,
32.79, 57.01, 119.40, 122.35, 128.95, 129.62,
129.71, 130.15, 133.49, 140.50, 141.36, 142.53,
167.05, 167.76, 172.51; anal. calc. for
$C_{23}H_{26}F_3N_3O_6S_2$, C: 49.20, H: 4.67, N: 7.48; found, C:
49.14 H: 4.71, N: 7.42.

EXAMPLE 5

HCl·cysteine-4-amino-2-phenylbenzoyl methionine methyl ester FTI274

5

N-BOC-S-trityl-cysteine-4-amino-2-phenylbenzoyl methionine methyl ester (0.06 g, 0.075 mmol) was dissolved into methanol (2 ml) and to it was added $HgCl_2$ (0.04 g) in methanol (1 ml). The reaction was carried out as described above to yield 15.7 mg of slightly impure compound by HPLC.

10

mp 130-132°C; ^1H NMR (CD_3OD) 1.72-1.84 (1H, m), 1.90-2.24 (6H, m), 3.05 (1H, dd, J=14.6 Hz, 8.5 Hz), 3.19 (1H, dd, J=14.6 Hz, 3.6 Hz), 3.69 (3H, s), 4.22 (1H, dd, J=.5 Hz, 3.6 Hz), 4.48-4.53 (1H, m), 7.33-7.43 (5H, m), 7.51 (1H, d, J=8.9 Hz),

15

7.70-7.72 (2H, m); ^{13}C NMR (CD_3OD) 15.04, 26.36, 30.88, 31.36, 52.85, 53.05, 56.93, 119.42, 122.38, 128.88, 129.55, 129.73, 130.05, 133.17, 140.55, 141.32, 142.52, 166.92, 172.61, 173.58; anal. calc. for $C_{24}H_{29}ClN_3O_6S2·2H_2O$, C: 51.20, H: 5.86, N:

20

8.14; found, C: 51.23 H: 5.60, N: 8.22.

EXAMPLE 6

SYNTHESIS OF FTI-275

A. 2-bromo-4-nitrobenzoic acid

2-bromo-4-nitrotoluene (5.00 g, 23.14 mmol)

25

was dissolved into pyridine (23 ml) and water (46 ml).· The heterogeneous mixture was heated to 60 ∞C and $KMnO_4$ (18.29 g, 115.7 mmol) was added carefully. The mixture was then heated under reflux overnight. The reaction mixture was

30

filtered and washed with boiling water. The solution was then made acidic and extracted into ethyl acetate, dried over Na_2SO_4, and the solvent was removed in vacuo. A crude NMR revealed remaining starting material so the solid was taken

35

up in NaOH and washed with hexanes. The aqueous phase was made acidic and the product was

extracted into ethyl acetate. The ethyl acetate
fractions were combined and dried over Na_2SO_4 and
the solvent was removed in vacuo to yield 3.72 g
(65.4%). mp 158-160°C; ^1H NMR (CD_3OD) 7.81 (1H, d,
J=8.5 Hz), 8.08 (1H, d, J=8.5 Hz), 8.30 (1H, s);
^{13}C NMR (CD_3OD) 121.96, 122.75, 129.36, 132.24,
139.52, 149.54, 167.75; anal. calc. for $C_7H_4BrNO_4$ +
0.1 ethyl acetate, C: 34.88, H: 1.90, N: 5.50;
found, C: 34.68, H: 1.86, N: 5.82.

B. 3,5-dimethylphenyl boronic acid

Mg turnings (1.44 g, 59.43 mmol) were covered
with dry THF (18.8 ml) in a dried, N_2 filled flask
fitted with an addition funnel and reflux
condenser. To this was added 5-bromo-m-xylene (10
g, 54.03 mmol) in THF (15 ml) after initiation of
the Grignard reaction. The addition was carried
out over several minutes and the reaction mixture
was heated at reflux for 1-2 h until most of the
Mg had reacted. The reaction mixture was then
cooled and transferred to an addition funnel
fitted to a N_2 filled flask containing triisopropyl
borate (24.9 ml) at -70 °C. The dropwise addition
was carried out over several minutes and the
mixture warmed to room temperature and stirred
overnight. The grey solution was poured onto 2 M
HCl and immediately turned yellow. The solution
was extracted into Et_2O and the Et_2O fractions were
combined, dried over $MgSO_4$ and the solvent was
removed in vacuo to yield 2.41 g (29.7%). mp 249-
251°C; ^1H NMR ($CDCl_3$) 2.44 (6H, s), 7.23 (1H, s),
7.84 (2H, s); ^{13}C NMR (CD_3OD) 21.36, 133.28,
134.39, 137.48.

C. 4-nitro-2-(3,5-dimethylphenyl)benzoic acid

2-bromo-4-nitrobenzoic acid (0.50 g, 2.03
mmol) and 3,5-dimethylphenyl boronic acid (0.34 g,

2.23 mmol) were dissolved into anhydrous DMF
(dimethylformamide) (25 ml) under nitrogen. To
this mixture was added Cs_2CO_3 (1.66 g, 5.08 mmol)
followed by $Pd(Ph_3P)_4$ (0.12 g, 5%). The mixture
5   was heated at 100°C overnight. The solution was
poured onto 1N HCl and extracted into Et_2O. It was
dried over $MgSO_4$ and the solvent was removed in
vacuo. The crude material was chromatographed on
silica gel using a 9:1 mixture of hexanes and
10   ethyl acetate to yield 0.34 g (61.7%) of pure
product. ^1H NMR ($CDCl_3$) 2.36 (6H, s), 6.99 (2H,
s), 7.07 (1H, s), 8.03 (1H, d, J=9.0 Hz), 8.23-
8.25 (2H, m); ^{13}C NMR ($CDCl_3$) 21.28, 121.68,
123.68, 125.74, 126.07, 130.22, 131.19, 131.31,
15   135.04, 138.21, 144.74, 170.75.

D.   4-nitro-2-(3,5-dimethylphenyl)benzoyl
methionine methyl ester

        4-nitro-2-(3,5-dimethylphenyl)benzoic acid
(0.15 g, 0.55 mmol), methionine methyl ester
20   hydrochloride salt (0.11 g, 0.55 mmol), EDCI
(0.11 g, 0.55 mmol), HOBT (0.07 g, 0.55 mmol) and
Et_3N (0.08 ml) in dry CH_2Cl_2 (2.2 ml) were reacted
and worked up according to the procedure for N-
BOC-4-aminobenzoyl methionine methyl ester in
25   Example 1. After recrystallization from ethyl
acetate and hexanes, 0.13 g (58.4%) of pure
product was isolated. mp 122-124°C; ^1H NMR ($CDCl_3$)
1.2-1.84 (1H, m), 1.85-1.97 (1H, m), 2.01 (3H, s),
2.05 (3H, t, J=7.7 Hz), 2.38 (6H, s), 3.70 (3H,
30   s), 4.67-4.74 (1H, m), 6.03 (1H, d, J=7.9 Hz),
7.05 (2H, s), 7.09 (1H, s), 7.84-7.87 (1H, m),
7.84-7.87 (1H, m), 8.23-8.26 (2H, m); ^{13}C NMR
($CDCl_3$), 15.20, 21.26, 29.22, 31.15, 51.79, 52.57,
122.07, 25.11, 126.27, 130.03, 130.53, 137.77,
35   138.82, 140.29, 141.56, 148.41, 167.14, 171.53.

E.   4-amino-2-(3,5-dimethylphenyl)benzoyl
methionine methyl ester

        4-nitro-2-(3,5-dimethylphenyl)benzoyl
methionine methyl ester   (0.11 g, 0.26 mmol) was
taken up in ethyl acetate (3.0 ml). To this
mixture was added $SnCl_2 \cdot 2H_2O$ (0.30 g, 1.30 mmol)
and the reaction was heated under nitrogen at
reflux for 6h. The mixture was worked up as
described for 4-amino-2-phenylbenzoyl methionine
methyl ester in Example 2, to give 0.15 g of a
yellow film that was wet with solvent. The
material was otherwise pure by NMR and was used
without further purification. ^1H NMR $(CDCl_3)$ 1.60-
1.70 (1H, m), 1.80-1.90 (1H, m), 1.99 (3H, s),
2.05 (2H, t, J=7.6 Hz), 2.33 (6H, s), 3.64 (3H,
s), 3.93 (2H, br s), 4.61-4.64 (1H, m), 5.82 (1H,
d, J=7.7 Hz), 6.49 (1H, d, J=2.3 Hz), 6.62 (1H,
dd, J=8.4 Hz, 2.4 Hz), 6.98 (2H, s), 7.00 (1H, s),
7.65 (1H, d, J=8.3 Hz); ^{13}C NMR $(CDCl_3)$ 15.08,
21.17, 29.28, 31.49, 51.70, 52.18, 113.30, 115.94,
123.55, 126.36, 129.32, 131.23, 138.15, 140.72,
141.92, 148.40, 168.45, 172.01.

F.   N-BOC-S-trityl-cysteine-4-amino-2-(3,5-
dimethylphenyl)benzoyl methionine methyl ester

        4-amino-2-(3,5-dimethylphenyl)benzoyl
methionine methyl ester (0.10g, 0.26 mmol) was
dissolved into dry CH_2Cl_2 (1.4 ml) and it was
allowed to stand. In another flask, N-BOC-S-
trityl-Cys (0.12 g, 0.26 mmol) was dissolved into
THF (4.4 ml) and was reacted with IBCF (0.03 ml)
and Et_3N (0.04 ml) as described above. The product
was worked up as described for N-BOC-S-trityl-
cysteine-4-aminobenzoyl methionine methyl ester in
Example 1 and chromatographed on silica gel using
a 1:1 hexanes and ethyl acetate elution mixture to
give 0.12 g (56.0%) of pure material. ^1H NMR

- 67 -

(CDCl$_3$) 1.33 (9H, s), 1.61-1.68 (1H, m), 1.73-1.91
(4H, m), 1.96 (2H, t, J=7.6 Hz), 2.24 (6H, s),
2.57- 2.64 (2H, m), 3.57 (3H, s), 4.00 (1H, br s),
4.54-4.58 (1H, m), 5.84 (1H, d, J=7.8 Hz), 5.97

5   (1H, br d), 6.90 (1H, s), 6.92 (2H, s), 7.18-7.22
(9H, m), 7.27-7.40 (7H, m), 7.55 (1H, m), 7.61
(1H, m), 8.58 (1H, br s); ^{13}C NMR (CDCl$_3$) 15.11,
21.20, 27.79, 29.25, 31.28, 51.70, 52.28, 54.08,
60.32, 71.45, 80.75, 118.01, 120.80, 126.38,

10   126.82, 127.98, 129.41, 129.87, 130.22, 138.11,
139.18, 139.79, 141.06, 144.17, 168.38, 169.04,
171.82.

G.   **TFA·Cysteine-4-amino-2-(3,5-
dimethylphenyl)benzoyl methionine FTI275**

15   N-BOC-S-trityl-cysteine-4-amino-2-(3,5-
dimethylphenyl)benzoyl methionine methyl ester
(0.12 g, 0.15 mmol) was placed into THF (0.9 ml)
and was reacted with 0.5 M of LiOH (0.6 ml) at 0 °C
as described above, followed by deprotection with

20   TFA (1.5 ml) and Et$_3$SiH (0.24 ml). Addition of
excess scavenger does not appear to affect the
result. The product was purified by preparative
HPLC to give 23.8 mg (27.3%). mp 135-138°C; ^{1}H NMR
(CDCl$_3$) 1.76-1.84 (1H, m), 2.00-2.17 (6H, m), 2.33

25   (6H, s), 3.05 (1H, dd, J=14.6 Hz, 7.3 Hz), 3.17
(1H, dd, J=14.6 Hz, J=4.9 Hz), 4.15 (1H, dd,
J=7.3, 4.9 Hz), 4.45-4.48 (1H, m), 7.02 (3H, s),
7.53 (1H, d, J=8.0 Hz), 7.66 (2H, m); ^{13}C (CD$_3$OD)
14.96, 21.51, 26.28, 30.91, 31.70, 53.03, 56.98,

30   119.27, 122.30, 127.52, 130.07, 130.57, 133.37,
139.28, 140.39, 141.29, 142.86, 166.89, 172.60,
174.81.

EXAMPLE 7

SYNTHESIS OF FTI-266

A.   4-amino-1-naphthoic acid

5   4-amino-1-naphthalenecarbonitrile (1.50 g,
8.91 mmol) was dissolved into a 50% KOH solution
(18 ml).   The heterogeneous solution was heated at
reflux for 2-3 days.   Once the solution became
homogenous and TLC showed no more starting
10   material, the deep red solution was cooled and
poured over 200 ml of water.   The solution was
then filtered and the acid was precipitated with
concentrated HCl.   The red crystals were filtered
and the filtrate was refiltered to give pink
15   crystals.   The first fraction was treated with
activated carbon to remove some of the red color.
1.51 g (90.6%) of product was obtained.   mp 169-
171°C; ^{1}H NMR (CD$_3$OD) 6.69 (1H, d, J=8.2 Hz), 7.38-
7.43 (1H, m), 7.48-7.54 (1H, m), 8.03 (1H, d,
J=8.5 Hz), 8.13 (1H, d, J=8.2 Hz), 9.09 (1H, d,
20   J=8.5 Hz); ^{13}C NMR (CD$_3$OD) 107.39, 114.61, 122.99,
123.92, 125.21, 127.40, 128.48, 135.04, 151.35,
171.44; HRMS calc. for C$_{11}$H$_7$NO$_2$, 187.0633; found,
187.0642.

B.   N-BOC-4-amino-1-naphthoic acid

25   4-amino-1-naphthoic acid (0.86 g, 4.61 mmol)
was dissolved into dioxane (9.2 ml) and 0.5 M NaOH
(9.2 ml).   Di-t-butyl dicarbonate (1.11 g, 5.07
mmol) was added and the mixture was stirred
overnight.   The reaction mixture was worked up as
30   described for N-BOC-4-aminobenzoic acid in Example
1 to give 0.76 g (56.7%) of reddish pink solid.
mp 194-195°C; ^{1}H NMR (CD$_3$OD) 1.56 (9H, s), 7.53-
7.62 (2H, m), 7.79 (1H, d, J=8.1 Hz), 8.12 (1H, d,
J=8.0 Hz), 8.22 (1H, d, J=8.18 Hz), 9.02 (1H, d,
35   J=8.9 Hz); ^{13}C NMR (CD$_3$OD), 26.68, 81.62, 119.06,
123.40, 124.57, 127.03, 127.37, 128.49, 128.77,

131.89, 133.76, 139.86, 155.95, 170.73; anal. calc. for $C_{17}H_{17}NO_4$, C: 66.90, H: 5.96, N: 4.88; found C: 66.49, H: 6.08, N: 4.79; m/ z (EI), 289; HRMS calc. for $C_{16}H_{17}NO_4$, 287.1158; found, 287.1151.

5   C. N-BOC-4-amino-1-naphthoyl methionine methyl ester   N-BOC-4-amino-1-naphthoic acid (0.46 g, 1.60 mmol), methionine methyl ester hydrochloride (0.35 g, 1.76 mmol), EDCI (0.43 g, 1.76 mmol), HOBT (0.24 g, 1.76 mmol) and Et_3N (0.27 ml) in

10   CH_2Cl_2 (6.4 ml) were reacted as described for N-BOC-4-aminobenzoyl methionine methyl ester in Example 1. After workup and recrystallization from ethyl acetate and hexanes, 0.44 g (63.6%) of pale pink crystals were obtained. mp 131-132°C; ^1H

15   NMR ($CDCl_3$) 1.57 (9H, s), 2.11-2.21 (4H, m), 2.29-2.41 (1H, m), 2.65 (2H, t, J=7.1 Hz), 3.83 (3H, s), 4.99-5.06 (1H, m), 6.68 (1H, d, J=8.0), 7.02 (1H, s), 7.56-7.59 (2H, m), 7.69 (1H, d, J=7.9 Hz), 7.87-7.90 (1H, m), 8.02 (1H, d, J=7.9 Hz),

20   8.44-8.48 (1H, m); ^{13}C NMR ($CDCl_3$) 15.56, 28.31, 30.19, 31.65, 52.06, 52.64, 81.17, 115.82, 120.18, 125.79, 126.37, 126.53, 127.18, 131.02, 135.65, 152.93, 169.04, 172.40; HRMS calc. for $C_{22}H_{28}N_2O_5S$, 432.1719; found 432.1702; m/ z (FAB) 433 (M+1).

25   D. HCl·4-amino-1-naphthoyl methionine methyl ester

       N-BOC-4-amino-1-naphthoyl methionine methyl ester (0.57 g, 1.31 mmol) was deprotected with HCl/ ether to yield 0.31 g (64.1%) of white solid.

30   mp 178-181°C; ^1H NMR (CD_3OD) 2.08-2.16 (4H, m), 2.20-2.30 (1H, m), 2.57-2.75 (2H, m), 3.82 (3H, s), 4.87-4.91 (1H, m), 7.59 (1H, d, J=7.5 Hz), 7.67 (1H, d, J=7.5 Hz), 7.71-7.80 (2H, m), 8.03 (1H, dd, J=7.1 Hz, 2.0 Hz), 8.35 (1H, dd, J=6.8

35   Hz, 1.8 Hz); ^{13}C NMR (CD_3OD) 15.23, 31.40, 53.01,

53.33, 119.90, 122.20, 126.15, 127.41, 127.77,
129.09, 129.31, 131.50, 132.33, 135.64, 171.77,
173.83; m/ z (FAB), 369 (M+1).

E. **N-BOC-S-trityl-cysteine-4-amino-1-naphthoyl**
5   **methionine methyl ester**

N-BOC-S-trityl-Cys (0.31 g, 0.67 mmol) in dry
THF (11.2 ml) was reacted with Et_3N (0.10 ml) and
IBCF (0.10 ml, 0.74 mmol) at -10 °C as described
above. HCl·4-amino-1-naphthoyl methionine methyl
10 ester (0.25 g, 0.67 mmol) in dry CH_2Cl_2 (3.5 ml)
was added and the mixture was stirred overnight
under nitrogen. The mixture was worked up as
described for N-BOC-S-trityl-cysteine-4-
aminobenzoyl methionine methyl ester in Example 1,
15 and the crude material was chromatographed on
silica gel using a 2:1 mixture of hexanes and
ethyl acetate to give 0.20g (37.5 %) of pure
product. ^1H NMR ($CDCl_3$) 1.48 (9H, s), 2.10-2.20
(4H, m), 2.30-2.37 (1H, m), 2.63 (2H, t, J=7.4),
20 2.74 (1H, J=12.9 Hz, J=5.3 Hz), 2.90 (1H, J=12.9
Hz, 6.2 Hz), 3.81 (3H, s), 4.96-5.03 (2H, m), 6.77
(1H, d, J=8.0 Hz), 7.18-7.33 (11H, m), 7.44-7.56
(7H, m), 7.60 (1H, d, J=7.7 Hz), 7.88 (1H, d,
J=8.0 Hz), 8.00 (1H, d, J=7.1 Hz), 8.37 (1H, d,
25 J=8.4 Hz), 8.94 (1H, br s); ^{13}C NMR ($CDCl_3$) 15.23,
26.52, 31.41, 31.50, 52.98, 53.31, 56.79, 68.15,
122.52, 123.54, 126.16, 126.99, 128.03, 128.39,
129.52, 132.30, 134.04, 135.24, 168.08, 172.38,
173.94.

30 F. **TFA·cysteine-4-amino-1-naphthoyl methionine,**
FTI-270

N-BOC-S-trityl-cysteine-4-amino-1-naphthoyl
methionine methyl ester (83.3 mg, 0.11 mmol) was
taken up in THF (0.7 ml) and to this mixture was
35 added 0.5 M LiOH (0.43 ml) at 0°C. The mixture

was stirred at 0°C for 35 minutes. The solvent
was removed in vacuo using a cold water bath. The
residue was worked up as described for
TFA·cysteine-4-amino-3-methylbenzoyl methionine in

5   Example 2, and 74.1 mg of the free acid was
obtained. This was then dissolved into CH_2Cl_2 (1
ml) and Et_3SiH (0.015 ml) was added followed by TFA
(1 ml). The reaction mixture was stirred at room
temperature for 1 h and worked up as further

10   described for TFA·cysteine-4-amino-3-methylbenzoyl
methionine in Example 2. After lyophilization,
42.4 mg of crude material was obtained which was
then purified on the HPLC using 0.1% TFA in water
and acetonitrile. mp 121-125°C; $[\alpha]^{25}_D=+2.4°$

15   (c=0.8, H_2O); ^1H NMR (CD_3OD) 2.03-2.13 (4H, m),
2.22-2.36 (1H, m), 2.59-2.74 (2H, m), 3.16-3.33
(2H, m), 4.42 (1H, m), 4.84-4.89 (1H, m), 7.57-
7.61 (2H, m), 7.64 (1H, d, J=7.7 Hz), 7.70 (1H, d,
J=7.7 Hz), 8.08-8.11 (1H, m), 8.29-8.32 (1H, m),

20   8.98 (1H, d, J=7.7 Hz); ^{13}C NMR (CD_3OD) 15.19,
26.45, 31.50, 31.63, 53.20, 56.72, 122.52, 123.43,
126.43, 126.12, 127.02, 127.96, 128.32, 129.49,
132.27, 134.15, 135.12, 168.11, 172.41, 175.17;
anal. calc. for $C_{21}H_{23}F_3N_3O_6S_2$, C: 47.19, H: 4.34, N:

25   7.86; found, C: 46.53, H: 4.56, N: 7.59; Note:
difference for C is 0.65.

G. HCl·cysteine-4-amino-1-naphthoyl methionine
methyl ester FTI-270·HCl
       TFA·cysteine-4-amino-1-naphthoyl methionine

30   (0.12 g, 0.15 mmol) was dissolved in CH_3OH (4.3
ml). To this solution was added a solution of
$HgCl_2$ (0.23 g, 0.86 mmol) in CH_3OH (4.3 ml). The
procedure was continued as described above and
after HCl/ Et_2O precipitation and several

35   reprecipitations 31.0 mg (18.3 %) of pure white
material was obtained. mp sub 137°C, decomp 214-

- 72 -

215°C; $[\alpha]^{25}_D$=-32.0° (c=1 CH$_3$OH); ^{1}H NMR (CD$_3$OD) 2.12
(3H, s), 2.21-2.28 (1H, m), 2.57-2.73 (3H, m),
3.20-3.34 (2H, m), 3.82 (3H, s), 4.39-4.43 (1H,
m), 7.61-7.68 (3H, m), 7.78 (1H, d, J=7.7 Hz),
8.13-8.16 (1H, m), 8.28-8.32 (1H, m); ^{13}C (CD$_3$OD)
15.23, 26.52, 31.41, 31.50, 52.98, 53.31, 56.79,
122.52, 123.54, 126.16, 126.99, 128.03, 128.39,
129.52, 132.30, 134.04, 135.24, 168.08, 172.38,
173.94.

                          EXAMPLE 8

SYNTHESIS OF FTI-254

A. N-Boc-S-trityl cysteinal

      Triethylamine (2.22 mL, 16 mmoL) and N,O-
dimethylhydroxylamine hydrochloride (1.57 g, 16.1
mmol) were added to a solution of N-Boc-S-trityl
cysteine (7.44 g, 16 mmol) in 85 mL of methylene
chloride. This mixture was cooled in an ice bath
and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide
hydrochloride (EDCI, 3.08 g, 16.0 mmol) and HOBT
(2.17 g, 16 mmol) was added. The mixture was
stirred at 0°C for 1 hr and at room temperature
for a further 10 hr. The mixture was extracted
with methylene chloride and 0.5 N HCl. The
organic layer was washed consecutively with 0.5 N
HCl, concentrated NaHCO$_3$ and brine. The organic
layer was dried and evaporated. The residue was
purified by flash column chromatography (1.5 : 1 =
hexane : ethylacetate) to give a white foam (7.40
g, 91%). m.p. 59-60 °C (decomp). ^{1}H NMR (CDCl$_3$) δ
7.41 (m, 6H), 7.20-7.31 (m, 9H), 5.13 (d, 8.9 Hz,
1H), 4.76 (br s, 1H), 3.64 (s, 3H), 3.15 (s, 3H),
2.56 (dd, 4.7 and 12.1 Hz, 1H), 2.39 (dd, 7.8 and
12.1 Hz, 1H), 1.43 (s, 9H). ^{13}C NMR (CDCl$_3$) δ
170.7, 154.9, 144.2, 129.3, 127.6, 126.4, 79.3,
66.4, 61.2, 49.5, 33.8, 31.8, 28.1. This
carboxyamide (2.02 g, 4.0 mmol) was dissolved in

30 mL of ether and cooled to -10 °C. Lithium
aluminum hydride (167 mg, 4.40 mmol) was added and
the mixture was stirred for 15 min under the
nitrogen.   Then 40 mL of 0.5 N HCl was added and
5   the solution was extracted with ether.   The ether
layer was washed with 0.5 N HCl and dried.   The
evaporation of solvents gave a white foam (1.80 g)
which was used for further reaction without
purification.   The ^{1}H NMR spectrum of this compound
10   was complex. The percentage of the aldehyde was
about 65-70%, which was calculated according to
the integration of the sharp singlet (δ 9.17) and
the trityl peak (δ 7.40, m, 6H; 7.28, m, 9H).
Lowering the temperature to -45°C did not improve
15   the aldehyde percentage.

B.   4-N-[2(R)-tert-Butoxycarbonylamino-3-
triphenylmethylthiopropyl]aminobenzoyl methionine
methyl ester.

One equivalent of N-Boc-S-trityl cysteinal in
20   10 mL of methanol was added to a solution of 4-
aminobenzoyl methionine methyl ester hydrochloride
(1.7836 g, 5.6 mmol) in 60 mL of methanol and 4 mL
of glacial acetic acid.   Sodium cyanoboronhydride
(0.528 g, 8.40 mmol) was added to this deep
25   colored solution at 0 °C. The mixture was stirred
at room temperature for 15 hr.   After the
evaporation of solvents, the residue was extracted
with ethyl acetate and concentrated sodium
bicarbonate.   The organic phase was dried and the
30   solvents were evaporated. The residue was purified
through flash column chromatography (ethyl acetate
/ hexane = 1:1) to give a pure desired product
(2.52 g, 65%). ^{1}H NMR (CDCl$_3$) δ 7.63 (d, 8.6 Hz,
2H), 7.43 (m, 6H), 7.21-7.32 (m, 9H), 6.73 (d, 7.6
35   Hz, 1H, Met amide), 6.50 (d, 8.6 Hz, 2H), 4.91
(ddd, 5.1 Hz, 5.3 Hz and 7.6 Hz, 1H, Met α H),

- 74 -

4.59 (d, 8.9 Hz, 1H, Boc amide), 4.25-4.40 (br, 1H, NHPh), 3.80 (m, 1H, Cys α H), 3.78 (s, 3H, OCH$_3$), 3.09 (d, 6.3 Hz, 2H, CH$_2$NH), 2.55-2.60 (m, 2H, CH$_2$SCPh$_3$), 2.46 (d, 5.0 Hz, 2H, CH$_2$SCH$_3$), 2.23-

5   2.28 (m, 1H, Met CH$_2$), 2.07-2.12 (m, 1H, Met CH$_2$), 2.09 (s, 3H, SCH$_3$), 1.43 (s, 9H, Boc).

C. 4-N-[2(R)-Amino-3-mercaptopropyl]aminobenzoyl methionine methyl ester.

The fully protected 4-N-[2(R)-tert-

10   Butoxycarbonylamino-3 triphenylmethylthiopropyl] amino-benzoyl methionine methyl ester (1.31 g, 1.83 mmol) was dissolved into 20 mL of methanol. To this solution was added mercuric chloride (1.09 g, 4.04 mmol) in 5 mL of methanol. The mixture

15   was refluxed for 20 min and then cooled down. The clear solution was removed and the solid precipitate was washed with 5 mL of methanol. The solid was dried and then suspended in 15 mL of methanol. The suspension was stirred and reacted

20   with hydrogen sulfide gas for 1 hr. The black precipitate was removed by centrifugation. The clear solution was evaporated to dryness. The residue was dissolved in 6 mL of methylene chloride followed by the addition of 20 mL of 3N

25   HCl in ether. The white precipitate was filtered and dried to give a hydrochloride salt of the desired product (0.60 g, 73%). ^{1}H NMR (CD$_3$OD) δ 7.73 (d, 8.8 Hz, 2H), 6.75 (d, 8.8 Hz, 2H), 4.74 (dd, 4.9 Hz and 4.3 Hz, 1H, Met α H), 3.72 (s, 3H,

30   OCH$_3$), 3.43-3.59 (m, 3H, CH$_2$NH and Cys α H), 2.93 (dd, 3.9 Hz and 14.4 Hz, 1H, CH$_2$SH), 2.81 (dd, 5.2 Hz and 14.5 Hz, 1H, CH$_2$SH), 2.49-2.66 (m, 2H, CH$_2$SCH$_3$), 2.07-2.20 (m, 2H, Met CH$_2$), 2.10 S, 3H, sCH$_3$).

D.   4-N-[2(R)-Amino-3-mercaptopropyl]aminobenzoyl methionine

The fully protected peptide 4-N-[2(R)-tert-Butoxycarbonylamino-3-triphenylmethylthiopropyl]-aminobenzoyl methionine methyl ester (567 mg, 0.79 mmol) was dissolved into 3.0 mL of 0.5 N lithium hydroxide and 3.0 mL of tetrahydrofuran. The mixture was stirred at 0°C for 1 hr. After the evaporation of solvents, the residue was dissolved in water and extracted with methylene chloride and 1N hydrochloric acid. The organic phase was dried and the solvents were evaporated. The residue was dissolved in a mixture of 1mL of methylene chloride and 2 mL of trifluoroacetic acid. Triethylsilane was added dropwise until the deep brown color disappeared. The mixture was kept at rt for 1 hr. The solvents were evaporated and the residue was dried. This residue was dissolved in 1 mL of 1.7N HCl in acetic acid followed by the addition of 20 mL of 3N HCl in ether. The white precipitate was filtered and dried to give a hydrochloride salt of the desired product (159 mg, 46%). Analytical HPLC showed purity over 98%. ^{1}H NMR (CD$_3$OD) δ 7.74 (d, 8.7 Hz, 2H), 6.75 (d, 8.7 Hz, 2H), 4.73 (dd, 4.5 Hz and 4.7 Hz, 1H, Met α H), 3.45-3.58 (m, 3H, CH$_2$NH and Cys α H), 2.93 (dd, 4.5 Hz and 14.6 Hz, 1 H, CH$_2$SH), 2.80 (dd, 5.3 Hz and 14.6 Hz, 1H, CH$_2$SH), 2.53-2.64 (m, 2H, CH$_2$SCH$_3$), 2.15-2.23 (m, 1H, Met CH$_2$), 2.07-2.13 (m, 1H, Met CH$_2$), 2.10 (s, 3H, SCH$_3$).

EXAMPLE 9

Synthesis of PTI-284

A.   4-Nitro-2-phenylbenzoyl-[1'(S)-methoxycarbonyl-3'-methylsulfonyl]propyl amide

4-nitro-2-phenylbenzoyl methionine methyl ester (525 mg, 1.28 mmol), N-methylmorpholine

oxide (453 mg, 3.87 mmol) and 0.5 mL of osmium
tetroxide (2.5 wt.% solution in tert-butanol) were
added to a mixture of 40 mL of acetone and 10 mL
of water. The mixture was stirred at rt

5   overnight. After the addition of excess sodium
sulfite, the reaction mixture was extracted with
ethyl acetate and washed with concentrated sodium
bicarbonate. The organic phase was dried and the
solvents were evaporated to give a solid (570 mg,

10   100%). ^{1}H NMR (CDCl$_3$) δ 8.29 (d, 7.7 Hz, 1H), 8.25
(s, 1H), 7.83 (d, 7.7 Hz, 1H), 7.43-7.55 (m, 5H),
6.15 (d, 7.3 Hz, 1H, Met amide), 4.68 (ddd, 5.0
Hz, 5.1 Hz and 7.3 Hz, 1H, Met α H), 3.70 (s, 3H,
OCH$_3$), 2.85 (s, 3H, SCH$_3$), 2.69-2.81 (m, 1H,

15   CH$_2$SO$_2$), 2.58-2.66 (m, 1H, CH$_2$SO$_2$), 2.21-2.33 (m,
1H, Met CH$_2$), 1.96-2.08 (m, 1H, Met CH$_2$).

B.   4-N-[2(R)-tert-Butoxycarbonylamino-3-
triphenylmethylthiopropyl]amino-2-phenylbenzoyl-
[1'(S)-methoxycarbonyl-3'-methylsulfonyl]propyl

20   amide   The 4-Nitro-2-phenylbenzoyl-[1'(S)-
methoxycarbonyl-3'-methylsulfonyl]propyl amide
(430 mg, 1.02 mmol) was dissolved in 20 mL of
methanol. A catalytic amount of 5% palladium on
carbon was added and the mixture was hydrogenated

25   at 40 PSI for 1.5 hr. The mixture was filtered and
the filtrate was evaporated to dryness to give 4-
amino product (400 mg, 100%). ^{1}H NMR (CD$_3$OD) δ 7.70
(d, 8.0 Hz, 1H), 7.38-7.47 (m, 7H), 4.53 (dd, 4.6
Hz and 4.8 Hz, 1H, Met α H), 3.72 (s, 3H, OCH$_3$),

30   2.89 (s, 3H, SO$_2$CH$_3$), 2.79-2.85 (m, 1H, CH$_2$SO$_2$),
2.58-2.68 (m, 1H, CH$_2$SO$_2$), 2.19-2.29 (m, 1H, Met
CH$_2$), 1.93-2.04 (m, 1H, Met CH$_2$). This amine was
dissolved in 15 mL of methanol and 0.8 mL of
acetic acid. One equivalent of N-Boc-S-trityl

35   cysteinal was added followed by the addition of
sodium cyanoboronhydride (97 mg, 1.5 eq). The

mixture was stirred at rt overnight. After the
evaporation of solvents, the residue was extracted
with ethyl acetate and concentrated sodium
bicarbonate. The organic phase was dried and

5   solvents were evaporated. The residue was
purified through flash column chromatography
(ethyl acetate / hexane / methanol = 15:15:2) to
give a pure product (500 mg, 60%). ^{1}H NMR (CDCl$_3$) δ
7.64 (d, 8.5 Hz, 1H), 7.37-7.46 (m, 11H), 7.18-

10   7.33(m, 9H), 6.53 (d, 8.5 Hz, 1H), 6.34 (s, 1H),
5.74 (d, 7.5 Hz, 1H, Met amide), 4.64 (ddd, 4.9
Hz, 5.1 Hz and 7.5 Hz, 1H, Met α H), 4.55 (d, 7.5
Hz, 1H, Boc amide), 4.26 (br, 1H, NHPh), 3.79 (m,
1H, Cys α H), 3.68 (s, 3H, OCH$_3$), 3.10 (t, 5.7 Hz,

15   2H, CH$_2$NHPh), 2.84 (s, 3H, SO$_2$CH$_3$), 2.62-2.82 (m,
2H, CH$_2$SO$_2$), 2.45 (d, 2H, CH$_2$SCPh$_3$), 2.19-2.27 (m,
1H, Met CH$_2$), 1.84-1.95 (m, 1H, Met CH$_2$), 1.41 (s,
9H).

        C.   4-N-[2(R)-Amino-3-mercaptopropyl]amino-2-
20   phenylbenzoyl-[1'(S)-methoxycarbonyl-3'-
        methylsulfonul]propyl amide, FTI-284
                The fully protected peptide 4-N-[2(R)-tert-
        Butoxycarbonylamino-3-triphenylmethyl-
        thiopropyl]amino-2-phenylbenzoyl-[1'(S)-

25   methoxycarbonyl-3'-methylsulfonyl]propyl amide(277
        mg, 0.33 mmol) was dissolved into 5 mL of
        methanol. To this solution was added mercuric
        chloride (229 mg, 2.50 eq) in 2 mL of methanol.
        The mixture was refluxed for 20 min. The

30   precipitate was dried and then suspended in 10 mL
        of methanol. This mixture was reacted with
        hydrogen sulfide gas. The reaction mixture was
        centrifuged and the clear solution was evaporated.
        The residue was dissolved in 2 mL of methylene

35   chloride followed by the addition of 20 mL of 3N
        HCl in ether. The white precipitate was collected

and dried to give a hydrochloride salt of the
desired product (165 mg, 89%). ^{1}H NMR (CD$_3$OD) δ
7.44 (d, 8.4 Hz, 1H), 7.32-7.40 (m, 5H), 6.77 (d,
8.4 Hz, 1H), 6.68 (s, 1H), 4.45 (dd, 4.5 Hz and
4.7 Hz, 1H, Met α H), 3.69 (s, 3H, OCH$_3$), 3.40-3.57
(m, 3H, CH$_2$NHPh and Cys α H), 2.78-2.96 (m, 3H,
CH$_2$SH and CH$_2$SO$_2$), 2.89 (S, 3H, SO$_2$CH$_3$), 2.60-2.69
(m, 1H, CH$_2$SO$_2$), 2.15-2.24 (m, 1H, Met CH$_2$), 1.91-
2.02 (m, 1H, Met CH$_2$).

EXAMPLE 10

Synthesis of FTI-277

A.   4-N-[2(R)-tert-Butoxycarbonyl-3-
triphenylmethylthiopropyl]amino-2-phenylbenzoyl
methionine methyl ester

    The coupling of 4-amino-2-phenylbenzoyl
methionine methyl ester (3.88 g, 10 mmol) with one
equivalent of N-Boc-S-trityl cysteinal in the
presence of 1.5 equivalent of sodium
cyanoboronhydride gave a crude mixture which was
purified through flash column chromatography
(ethyl acetate / hexane = 1:1) to give a pure
desired product (5.83 g, 74%). ^{1}H NMR (CDCl$_3$) δ
7.65 (d, 8.5 Hz, 1H), 7.32-7.45 (m, 11H), 7.18-
7.30 (m, 9H), 6.50 (d, 8.5 Hz, 1H), 6.33 (s, 1H),
5.65 (d, 7.6 Hz, 1H, Met amide), 4.62 (ddd, 5.0
Hz, 5.2 Hz and 7.6 Hz, 1H, Met α H), 4.54 (d, 8.1
Hz, Boc amide), 4.18 (br, 1H, NHPh), 3.78 (m, 1H,
Cys α H), 3.64 (s, 3H, OCH$_3$), 3.10 (t, 6.1 Hz, 2H,
CH$_2$NHPh), 2.45 (d, 5.0 Hz, 2H, CH$_2$SCPh$_3$), 2.04-2.10
(m, 2H, CH$_2$SCH$_3$), 2.00 (s, 3H, SCH$_3$), 1.81-1.92 (m,
1H, Met CH$_2$), 1.61-1.70 (m, 1H, Met CH$_2$), 1.40 (s,
9H). ^{13}C NMR (CDCl$_3$) δ 172.0, 168.3, 155.7, 149.4,
144.3, 141.6, 141.1, 131.3, 129.5, 128.7, 128.5,
127.9, 127.7, 126.8, 122.6, 113.6, 111.3, 79.8,
67.1, 52.2, 51.7, 49.5, 47.2, 34.3, 31.6, 29.4,
28.3, 15.2.

B.   4-N-[2(R)-Amino-3-mercaptopropyl]amino-2-phenylbenzoyl methionine methyl ester, FTI-277.

The fully protected peptide 4-N-[2(R)-tert-Butoxycarbonyl-3-triphenylmethyl-thiopropyl]amino-2-phenylbenzoyl methionine methyl ester (1.57 g, 2.0 mmol) was first reacted with mercuric chloride (1.36 g, 5.0 mmol) and then reacted with hydrogen sulfide gas in methanol to give a hydrochloride salt of the desired product (0.808 g, 84%). Analytical HPLC showed purity over 98%. $[\alpha]^{25}_D$ = -12.1° (c=0.008, CH$_3$OH). ^{1}H NMR (CD$_3$OD) δ 7.42 (d, 8.3 Hz, 1H), 7.30-7.38 (m, 5H), 6.78 (d, 8.3 Hz, 1H), 6.71 (s, 1H), 4.47 (dd, 4.2 Hz and 5.1 Hz, 1H, Met α H), 3.68 (s, 3H, OCH$_3$), 3.44-3.54 (m, 3H, CH$_2$NHPh and Cys α H), 2.94 (dd, 4.1 Hz and 14.6 Hz, 1H, CH$_2$SH), 2.81 (dd, 5.0 Hz and 14.6 Hz, 1H, CH$_2$SH), 2.12-2.22 (m, 1H, CH$_2$SCH$_3$), 2.03-2.10 (m, 1H, CH$_2$SCH$_3$), 2.00 (s, 3H, SCH$_3$), 1.90-1.97 (m, 1H, Met CH$_2$), 1.73-1.82 (m, 1H, Met CH$_2$). ^{13}C NMR (CD$_3$OD) δ 173.7, 173.4, 150.7, 143.5, 142.3, 131.2, 129.8, 129.5, 128.6, 125.6, 115.6, 112.2, 53.7, 53.2, 52.8, 45.0, 31.3, 30.8, 25.3, 15.0.

EXAMPLE 11

Synthesis of FTI-276

4-N-[2(R)-Amino-3-mercaptopropyl] amino-2-phenylbenzoyl methionine

The fully protected peptide 4-N-[2(R)-tert-Butoxycarbonyl-3-triphenylmethylthiopropyl]-amino-2-phenylbenzoyl methionine methyl ester (2.36 g, 3 mmol) was first reacted with lithium hydroxide and then with trifluoroacetic acid to give a crude product (1.30 g, 77% yield, 85% purity shown by HPLC) which was further purified through preparative HPLC to give a pure product (0.98 g, 75%). $[\alpha]^{25}_D$ = -13.6° (c=0.005, CH$_3$OH). ^{1}H NMR (CD$_3$OD) δ 7.44 (d, 8.4 Hz, 1H), 7.30-7.41 (m, 5H),

6.75 (d, 8.4 Hz, 1H), 6.68 (s, 1H), 4.43 (dd, 4.2

Hz and 5.1 Hz, 1H, Met α H), 3.44-3.58 (m, 3H,

CH₂NHPh and Cys α H), 2.95 (dd, 4.4 Hz and 14.5 Hz,

1H, CH₂SH), 2.83 (dd, 5.0 Hz and 14.5 Hz, 1H,

5   CH₂SH), 2.14-2.23 (m, 1H, CH₂SCH₃), 2.05-2.11 (m,

1H, CH₂SCH₃), 2.00 (s, 3H, SCH₃), 1.91-1.99 (m, 1H,

Met CH₂), 1.72-1.81 (m, 1H, Met CH2). ¹³C NMR

(CD₃OD) δ 176.4, 173.5, 150.4, 143.0, 141.5, 131.0,

129.7, 129.4, 128.9, 124.6, 115.0, 112.2, 53.3,

10   44.4, 30.8, 30.1, 24.9, 14.8.

        Other compounds of the invention (in

particular those of claims [[14-18) are

synthesizable by modifications of the procedure

described for the 2-phenyl-4-aminobenzoic acid

15   derivative of claim 3. In particular,

modifications of the Suzuki couping method will

allow the incorporation of an alkoxy-, chloro,

bromo or methyl substituted phenyl group onto the

4-aminobenzoic acid spacer. As with the

20   unsubstituted derivative, 4-nitro-2-bromotoluene

will be coupled with the corresponding substituted

phenyl boronic acid derivative (alkoxyphenyl or

chloro-, bromo- or methylphenylboronic acid) under

paladium catalyzed conditions. The appropriately

25   substituted 2-(substituted) phenyl-4-nitro toluene

derivative will be incorporated into the

peptidomimetic synthesis as described for the 2-

phenyl case.

        In a similar way the precursor to the 2-

30   naphthyl-, 2-thiophene-, 2-pyrrole-, and 2-

pyridyl-4-aminobenzoic acid spacers can be

prepared by reaction of 4-nitro-2-bromotoluene

with naphthalene-2-boronic acid, thiophene-2-

boronic acid, pyrrole-2-boronic acid, pyridine-

35   2,3- or 4-boronic acid.

EXAMPLE 12

FTase and GGTase I Activity Assay

FTase and GGTase I activities from 60,000 X g
supernatants of human Burkitt lymphoma (Daudi)
cells (ATCC, Rockville, MD, USA) were assayed as
described previously for FTase (41). Briefly, 100
μg of the supernatant was incubated in 50 mM Tris,
pH 7.5, 50 μM $ZnCl_2$, 20 mM KCl and 1 mM
dithiothreitol (DTT). The reaction was incubated
at 30°C for 30 min with recombinant Ha-Ras-CVLS (11
μM) and [^{3}H]FPP (625 nM; 16.3 Ci/mmol) for FTase,
and recombinant Ha-Ras-CVLL (5 μM) and
[^{3}H]geranylgeranylpyrophosphate (525 nM; 19.0
Ci/mmol) for GGTase I. The peptidomimetics were
mixed with FTase and GGTase before adding to the
reaction mixture.

EXAMPLE 13

Ras and Rap1A Processing Assay

H-RasF cells (45) were seeded on day 0 in 100
mm Dishes (costar) in Dulbecco's modified Eagles
medium (GIBCO) and allowed to grow to 40-60%
confluency. On days 1 and 2, cells were fed with
4 ml of medium per plate plus various concentra-
tions of FTI-277 or vehicle. On day 3, cells were
washed one time with ice cold PBS and were
collected and lysed by incubation for 30-60 min on
ice in lysis buffer (41). Lysates were cleared
(14,000 rpm, 4°C, 15 min) and supernatants
collected. Equal amounts of lysate were separated
on a 12.5% SDS-PAGE, transfered to nitrocellulose,
and a western blot performed using a anti-Ras
antibody (Y13-238, ATCC) or anti-Rap1A antibody
(Santa Cruz Biotechnology, Santa Cruz, CA).
Antibody reactions were visualized using
peroxidase-conjugated goat anti-rat IgG for Y13-
238 and peroxidase-conjugated goat anti-rabbit IgG

for Rap1A and an enhanced chemiluminescence
detection system (ECL; Amersham Corp.)

                    EXAMPLE 14

Co-immunoprecipitation of Raf and Ras

5   Cells were seeded on day 0 in 100 mm dishes
     in 10 ml Dulbecco's Modified Eagles Medium
     (GIBCO) supplemented with 10% calf serum (Hyclone)
     and 1% pen/strep (GIBCO). On days 1 and 2 cells
     were treated with FTI-277 (5 μM) or vehicle
10   (confluency of cells 40-60%). On day 3, cells
     were collected by centrifugation in ice cold PBS.
     Cell pellets were then resuspended in ice cold
     hypotonic buffer (10 mM Tris, pH 7.5, 5 mM $MgCl_2$, 1
     mM DTT, 1 mM PMSF) and cells were sonicated to
15   break up cell pellet to promote separation of
     cytosol and membrane. The cell suspension was
     then centrifuged at 2,000 rpm for 10 min to clear
     debris after which the supernatant was loaded in
     ultrocentrifuge tubes and spun for 30 min at
20   100,000 X g to SW Ti55 Rotor to separate membrane
     and cytosol fractions. The cytosol and membrane
     fractions were lysed on ice for 60 min in buffer
     containing 30 mM HEPES, pH 7.5, 1% TX-100, 10%
     glycerol, 10 mM NaCl, 5 mM MgCl2, 2 mM Na_3VO_4, 25
25   mM NaF, 1 mM EGTA, 10 μM soybean trypsin
     inhibitor, 25 μg/ml leupeptin, 10 μg/ml aprotinin,
     2 mM PMSF). The lysates were clarified by
     centrifugation. Equal amounts of cytosol and
     membrane fractions were immunoprecipitated using
30   50 μl of a 25% Protein-A Sepharose Cl-4B
     suspension (Sigma) with 1 μf/ml anti-c-Raf-1
     (SC133, Santa Cruz Biotechnology, Santa Cruz, CA).
     The samples were tumbled at 4°C for 60 min and then
     washed 5 times in 50 mM HEPES, pH 7.5, 100 mM
35   NaCl, 5 mM $MgCl_2$, 0.1% TX-100, 10% glycerol, 20 mM
     NaF. The final pellets were run on 12.5% SDS-

PAGE, transferred to nitrocellulose, and
immunoblotted for the presence of Ras using anti-
Ras antibody (Y13-238) and immunoblotted for the
presence of Raf (c-Raf-1, SC133, Santa Cruz
5   Biotechnology, Santa Cruz, CA). Detection was the
same as above for Ras and Rap1A processing.

                          EXAMPLE 15
        Detection of GTP and GDP bound to Ras (FTI-277)
               H-RasF cells were seeded and treated as above
10   for Ras/Raf interaction and Ras and Rap1A
        processing. On day 2, however, cells were labeled
        overnight with [^{32}P] orthophosphate at 100 μCi/mo
        (Amersham PBS13) in 10 ml DMEM-phosphate
        supplemented with 10% calf serum, 1 mg/ml BSA and
15   20 mM HEPES, pH 7.5. On day 3, the medium was
        removed and cells were washed one time in ice-cold
        PBS, scraped from the plate with a cell scraper,
        collected and centrifuged. The cell pellet was
        resuspended in ice-cold hypotonic buffer listed
20   above and the cytosol and membrane fractions were
        separated according to the above description for
        Ra/Raf association. The cytosol and membrane
        fractions were lysed on ice for 60 min in 50 mM
        Tris, pH 7.5, 5 mM MgCl$_2$, 1% Triton X-100 (TX-100),
25   0.5% DOC, 0.05% SDS, 500 mM NaCl, 1 mM EGTA, 10
        μg/ml aprotinin, 10 μg/ml soybean trypsin
        inhibitor, 25 μg/ml leupeptin, 1 mM DTT, 1 mg/ml
        BSA. Lysates were cleared and equal amounts of
        protein were immunoprecipitated using anti-Ras
30   antibody (Y13-259) along with 30 μl Protein A-
        Agarose goat anti-rat IgG complex (Oncogene
        Science) for 60 min at 4°C. Immunoprecipitates
        were washed 6 times in 50 mM HEPES, pH 7.5, 0.5 M
        NaCl, 0.1% TX-100, 0.0005 SDS, 5 mM MgCl$_2$, drained
35   using a syringe and bound nucleotide eluted in 12
        μl of 5 mM DTT, 5 mM EDTA, 0.2% SDS, 0.5 mM GDP

and 0.5 mM GTP at 68°C for 20 min. Immune
complexes were spun down quickly and 6 μl of the
supernatent was loaded onto PEI cellulose thin
layer chromatography plates (20 cm X 20 cm).

5   Nucleotides were separated by chromatography in 78
g/linter ammonium formate, 9.6% (v/v) concentrated
HCl. Plates were analyzed by autoradiogram.

EXAMPLE 16

Analysis of Raf-I Kinase Activity

10   Raf-1 kinase was assayed by determining the
ability of Raf to transfer phosphate from [γ-^{32}P]
ATP to a 19-mer peptide containing an
autophosphorylation site. Membrane and cytosol
fraction isolation and Raf immunoprecipitates were

15   washed three times with cold HEPES buffer and
twice with kinase buffer (50 mM Tris, pH 7.5, 150
mM NaCl, 12 mM MnCl$_2$, 1 mM DTT, 0.2% Tween 20.
Immune complex kinase assays were performed by
incubating immunoprecipitaes from membrane and

20   cytosol fractions in 96 μl of kinase buffer with
20 μCi of [γ-^{32}P]ATP (10 mCi/ml, Amersham) and 2
μl of the Raf-1 substrate peptide (1 mg/ml,
Promega) for 30 min at 25°C. The sequence of the
Raf-1 substrate peptide is IVQQFGFQRRASDDGKLTD.

25   The phosphorylation reaction was terminated by
spotting 50 μl aliquots of the assay mixture onto
Whatman P81 for 40 min in 0.5% orthophosphoric
acid and air dried. The amount of ^{32}P incorporated
was determined by liquid scintillation counting.

30   EXAMPLE 17

Inhibition of FTase by FTI-276 and other compounds

Fig. 1B shows that FTI-276 inhibited the
transfer of farnesyl from [^{3}H]FPP to recombinant H-
Ras-CVLS with an IC$_{50}$ of 500 pM. FTI-249, the
35   parent compound of FTI-276, inhibited FTase with

an IC_{50} of 200,000 pM. Thus, a phenyl ring at the
2 position of the benzoic acid spacer increased
inhibition potency of FTase by 400 fold confirming
our prediction of a significant hydrophobic pocket

5   within the CAAX binding site of FTase. This
extremely potent inhibitor was also highly
selective (100-fold) for FTase over the closely
related GGTase I (Fig. 1B). FTI-276 inhibited the
transfer of geranylgeranyl from [^{3}H]GG-PP to

10   recombinant H-Ras-CV11 with an IC_{50} of 50 nM (Fig.
1B). This 100-fold selectivity is superior to the
15-fold selectivity of the parent compound, FTI-
249. Data for a number of other compounds of
interest are shown in Table 1.

Table 1

Compound		PTase IC_{50} (nm)	GGTase I IC_{50} (nm)	GG/F
PTI				
232	CABAM	213	1200	6
260	3-Me-CABAM	825	9000	11
261	3-OMe-CABAM	2550	50000	20
270	CANAM	143	3150	22
				-
272	2-Ph-CABAM	5	267	53
274	2-Ph-CABAM-OMe	2050	30000	15
275	2-Xy-CABAM	405	400	1
249	red.CABAM	272	3967	15
254	red.CABAM-OMe	1000	19000	19
276	red.2-Ph-CABAM	0.5	57	114
277	red.2-Ph-CABAM-OMe	50	1600	32

EXAMPLE 18

Inhibition of Ras Processing by FTI-277

To facilitate cellular uptake, FTI-277, the methylester of FTI-276, was used in experiments to measure inhibition of Ras processing. H-RasF cells (NIH 3T3 cells transformed with oncogenic (61 leucine) H-Ras-CVLS (45) were treated with FTI-277 (0-50 μM) and the lysates blotted with anti-Ras or anti-Rap1A antibodies. As shown in Fig. 2A, concentrations as low as 10 nN inhibited Ras processing but concentrations as high as 10 μM did not inhbit processing of the geranylgeranylated Rap1A. FTI-277 inhibited Ras processing with an IC_{50} of 100 nM. In contrast, the IC_{50} of FTI-249 is 100 μM, and the most potent CAAX peptidomimetics previously reported inhibited

- 87 -

Ras processing at concentrations of 10 μM or
higher (44).

The selectivity of FTI-277 for farnesylation
but not geranylgeranylation processing is further
5   demonstrated in Fig 2B. H-RasGG cells (NIH 3T3
cells transformed with oncogenic (61 leucine) H-
Ras-CVLL (45) were treated with FTI-277.
Processing of RasGG was not affected, whereas that
of RasF was completely blocked. The processing of
10   endogenous Ras is also blocked in pZIPneo cells
(NIH 3T3 cells transfected with the same plasmid
as H-RasF and H Ras FF except the vector contained
no oncogenic Ras sequences) and Raf cells (NIH 3T3
cells transformed by an activated viral Raf (48)).

15   **Mechanism of disruption of Ras oncogenic**
signalling by FTI-277

Ras relays biological information from
tyrosine kinase receptors to the nucleus by
activation of a cacade of MAPKs (reviewed in 29-
20   31). Upon growth factor stimulation, Ras becomes
GTP bound and is then able to recruit the ser/thr
kinase c-Raf-1 to the plasma membrane where it is
activated. c-Raf-1 then phosphorylates and
activates MEK, a dual thr/tyr kinase, which
25   activates MAPK. Recently, epidermal growth factor
has been shown to induce association of Raf with
Ras (46).

In order to determine the mechanism by which
FTI-277 disrupts Ras oncogenic signaling, NIH 3T3
30   cells were transfected with activated (GTP-locked)
Ras and the effects of FTI-277 on the interaction
of Ras with its immediate effector, Raf, were
investigated. Various NIH 3T3 cell transfectants
(pZIPneo, H-RasF, and H-RasGG) were treated with
35   vehicle or FTI-277, membrane and cytosolic
fractions were isolated and immunoprecipitated

with anti-Raf antibody as described above. Raf
did not associate with Ras in pZIPneo cells which
did not contain GTP-locked Ras, as shown in Fig.
3. In contrast, H-RasF and H-RasGG cells contain
5   Ras/Raf complexes in the membrane, but not in the
cytosolic fractions, as shown in Fig. 3.
Treatment of these cells with FTI-277 resulted in
the accumulation of Ras/Raf complexes in the
cytosolic but not membrane fractions of H-RasF
10   cells, but not in the H-RasGG cells (Fig 3).
Thus, the disruption of Ras/Raf interaction at the
cell membrane and accumulation of these complexes
in the cytoplasm occurred only in Ras-F but not
Ras-GG cells, in agreement with the Ras processing
15   selectivity results of Fig. 2. Thus, these
results demonstrate that inhibition with FTI-277
results in the accumulation of non-farnesylated
cytosolic Ras that is capable of binding to Raf.
The fact that non-processed Ras can associate with
20   Raf in a non-membranous cytoplasmic environment
was confirmed by transfecting NIH 3T3 cells with a
GTP-locked Ras that lacks a farnesylated site and,
therefore, remains in the cytoplasm (Ras mutant
with a 61 leucine oncogenic mutation and a 186
25   serine mutation) and showing that these cells
contained only cytoplasmic Ras/Raf complexes when
immunoprecipitated with Raf and blotted with
antiRas antibodies (Fig. 3). In short,
farnesylation is not required for Ras to bind to
30   Raf.

EXAMPLE 19
Determination of nucleotide state of Ras
The fact that Raf binds Ras-GTP with much
higher affinity than Ras-GDP was used to determine
35   the nucleotide state of Ras in the cytoplasmic
Ras/Raf complexes, as described above. In Ras-F

cells, only membrane fractions contained GTP-
locked Ras, as shown in Fig 4A. Upon treatment
with FTI-277, however, the non-farnesylated
cytosolic Ras was found to be GTP bound. Thus,
5   binding of GTP to 61 leucine Ras does not require
Ras processing and subsequent plasma membrane
association. The ser/thr kinase activity of Raf
in Ras/Raf complexes was then determined by
immunoprecipitating Raf and assaying for its
10   ability to phosphorylate a 19-mer
autophosphorylated peptide. Fig. 4B shows that
oncogenic Ras-F induced activation of Raf in the
plasma membrane and that treatment with FTI-277
suppressed this activation. More importantly, the
15   cytoplasmic Ras/Raf complexes that were induced by
FTI-277 (Fig. 3) had basal levels of Raf kinase
activity that were comparable to those of the
parental NIH 3T3 cell line pZIPneo (Fig. 4B).
Taken together, Figures 3 and 4 demonstrate that
20   oncogenic transformation with GTP-locked Ras
results in the constitutive recruitment to the
plasma membrane and subsequent activation of Raf.
Furthermore, FTase inhibition by FTI-277
suppresses this activation by inducing the
25   accumulation of Ras/Raf complexes in the cytoplasm
where Ras is GTP-bound but Raf kinase is not
activated. The fact that Raf kinase is not
activated when bound to Ras in a non-membranous
environment is consistent with recent reports that
30   indicate that Raf activation requires an as yet to
be determined activating factor at the plasma
membrane (47).

        Experiments were then performed to
investigate the effects of FTI-277 on oncogenic
35   Ras activation of MAPK, a Raf downstream
signalling event (29-31). Oncogenic activation of
MAPK can be easily detected since activated MAPK

migrates slower in SDS-PAGE. Fig. 5A shows that
NIH 3T3 cells transfected with pZIPneo contain
only inactive MAPK but that upon transformation
with oncogenic H-Ras, MAPK is activated (Fig. 5A).

5   Pretreatment with FTI-277 results in a
concentration dependent inhibition of oncogenic
Ras activation of MAPK. Concentrations as low as
300 nM were effective and the block was complete
at 1 μM. Taken together, Figs. 3 and 5

10   demonstrate that at least 50% inhibition of Ras
processing is required for complete suppression of
MAPK activation but that less than a 100%
inhibition of Ras processing is required for
complete suppression of MAPK activation by Ras. A

15   series of NIH 3T3 cell lines transformed with
various oncogenes was used to determine whether
the inhibition of MAPK activation is due to
selectively antagonizing Ras function. Fig. 5B
shows that FTI-277 was able to block H-RasF but

20   not H-RasGG activation of MAPK. This is
consistent with its ability to inhibit H-RasF but
not H-RasGG processing (Fig. 2). Selectivity of
FTI-277 towards antagonizing Ras-dependent
activation of MAPK was substantiated by using NIH

25   3T3 cells where MAPK is constitutively activated
by transformation with the Raf oncogene. Fig.
5B shows that oncogenic Raf activation of MAPK is
not blocked by FTI-277 even though processing of
endogenous Ras was inhibited in these cells.

30   Similar results were also obtained with FTI-276
(Fig. 6). Taken together these results clearly
demonstrate that FTI-276 and FTI-277 are highly
effective and selective in disrupting contitutive
Ras-specific activation of MAPK.

35   Thus, FTI-277 is an extremely potent and
highly selective FTase inhibitor. This compound
inhibited Ras processing with concentrations as

low as 10 nM and processing was blocked at 1 μM.
The most potent inhibitor previously reported BZA-
5B, blocked Ras processing only at 150 μM (44).

EXAMPLE 20

5   Antitumor Efficacy and Selectivity of FTI-276 and
FTI-277   In order to demonstrate the efficacy of
these inhibitors as anticancer agents and show
that they can inhibit tumor growth of human tumors
which have multiple and complex genetic
10   alterations, antitumor efficacy experiments were
performed using a human tumor cell line. A
critical issue connected with the potential use of
the compounds of the invention is whether the
growth of human tumors which harbor K-Ras
15   mutations can be blocked. This is important for
further development of FTase inhibitors as
anticancer drugs since K-Ras mutations are most
common in human cancers and since K-Ras processing
is more difficult to inhibit than the processing
20   of the less prevalent H-Ras (1-3, 15).
Furthermore, the majority of human tumors have
multiple genetic alternations; notably a delation
in the tumor suppressor gene p53 is most
prevalent. It is therefore extremely important to
25   determine whether or not inhibition of Ras
function is sufficient to halt the growth of human
tumors which harbor K-Ras mutation as well as
deletions in p53.

To evaluate the antitumor efficacy of FTI-
30   276, a nude mouse xenograft model was used. In
this model, tumors from two human lung carcinoma
cell lines are implanted subcutaneously. One of
these (Calu-1) harbors a K-Ras oncogenic mutation
and has a deletion of the tumor suppressor gene
35   p53. The other human lung carcinoma (NCI-H180)
has no Ras mutations. Thirty two days after s.c.

implantation when the tumors reached sizes of 60
to 80 mm³, the mice were randomly separated into
control and treated groups (4 animals per group;
each animal had a tumor on both the right and the

5   left flank). Figure 7A shows that tumors from
control animals treated with saline once daily
starting on day 36 grew to an average size of 566
mm³ over a period of 64 days from tumor
implantation. In contrast, tumors treated once

10   daily with FTI-276 (50 mg/kg) grew very little and
the average tumor size was 113 mm³ (Fig. 7A). In
another experiment, FTI-277, the methylester of
FTI-276, inhibited the growth of Calu-I cells to
the same extent (Figure 8). Although the animals

15   were treated once daily with 50 mg/kg for 36 days
(total cumulative does of 1.8 g/kg), no weight
loss was observed and the animals appeared normal
with no evidence of gross toxicity. This lack of
toxicity was also observed in separate experiments

20   where the dose was escalated to 180 mg/kg once
daily. Thus, FTI-276 and FTI-277 essentially
blocked tumor growth of Calu-I carcinoma with no
evidence of gross toxicity.

         The effect of FTI-276 on the tumor growth of

25   another human lung carcinoma, NCI-H810, that does
not harbor an oncogenic Ras mutation was also
determined. Figure 7B shows that tumors from
animals treated with saline or FTI-276 grew at a
similar rate. Over a period of 14 days of

30   treatment the average tumor size of the control
and FTI-276 treated groups were 919 mm³ and 815
mm³, respectively. These results clearly
demonstrate that in contrast to Calu-1, NCI-H810
carcinomas were not sensitive to FTI-276 treatment

35   suggesting that FTI-276 inhibition of tumor growth
of human lung carcinomas is Ras-dependent.

Furthermore, FTI-276 inhibited tumor growth even
though Calu-1 does not express p53.

To further establish the selectivity of FTI-
276 to inhibit selectively Ras-dependent tumors,
5   the anti-tumor efficacy of FTI-276 and FTI-277
against H-RasF and Raf transformed NIH 3T3 in the
same nude mouse xenograft model was examined.
Figure 9 shows that a once daily injection of FTI-
276 or FTI-277 (50 mg/kg) inhibited tumor growth
10   of H-RasF transformed NIH 3T3 cells. In contrast,
an identical treatment regimen with FTI-276 and
FTI-277 had no effect on the growth of Raf-
transformed NIH 3T3 cells (Fig. 10), further
confirming the conclusion from the results of
15   Figures 7 and 8 that FTI-276 and FTI-277 are
selective for Ras-dependent tumors.

In addition, the question of whether FTI-276
inhibition of tumor growth correlated with
inhibition of Ras processing *in vivo* was
20   addressed. To so this, mice having subcutaneous
H-RasF cells were treated with various doses of
FTI-276 (0, 10, 50 and 100 mg/kg) and tumor size
and Ras processing in the HRasF tumors *in vivo*
were examined. Figure 11A shows that throughout
25   the 11 day treatment period, FTI-276 inhibited
tumor growth in a dose dependent fashion. The
tumor sizes at the end of 17 days were 2490 mm^3 for
saline, 1793 mm^3 for 10 mg/kg, 1226 mm^3 for 50
mg/kg and 624 mm^3 for 100 mg/kg treated animals.
30   To determine the levels of inhibition of Ras
processing, the animals were sacrificed 5 hrs
after the last injection, the tumors were excised
and processed for immunoblotting with anti-Ras
antibody as described in legend to Fig. 11.
35   Tumors from control animals contained only fully
processed Ras which migrates faster in SDS-PAGE
gels (Fig. 11B). As the dose of FTI-276 increases

from 10 to 100 mg/kg there was a progressive
accumulation of unprocessed Ras which was
paralleled by a decrease in the relative ratio of
fully processed Ras. Thus, the extent of tumor
5   growth inhibition correlated with the extent of
inhibition of Ras processing. Furthermore, the
inhibition of Ras processing in vivo was selective
in that FTI-276 did not inhibit Rap1A processing
even at 100 mg/kg.

10   **II. Farnesyltransferase Inhibitors of the type**
CΔ

Compounds of another major embodiment of the
invention are represented by formula II. Several
examples are shown in Figure 12. These and other
15   compounds of this embodiment may be prepared using
procedures which are conventional in the art. For
example, compounds 4 and 5 of Figure 12 may be
prepared by reductive amination of 4-amino-3'-
tert.butoxy-carbonyl biphenyl or 4-amino-4'-
20   tert.butoxy carbonyl biphenyl, respectively, with
N-Boc-S-trityl cysteinal followed by deprotection
with, for example, trifluoroacetic acid and
purification.

This embodiment of the invention is
25   illustrated but not limited by the following
examples:

<u>EXAMPLE 21</u>

The compound C-4ABA-Met of formula (2) (see
Figure 12) was prepared as described in reference
30   (27). The protected form of the peptidomimetic
(2a) was prepared through the reductive amination
of 4-aminobenzoyl methionine methyl ester and N-
Boc-S-trityl cysteinal in methanol solution
containing NaBH$_3$CN and 5% acetic acid. This
35   reaction gave the N-Boc-S-trityl, methyl ester of
(2a) with a yield of 65%. The protected

peptidomimetic was deesterified by LiOH in THF and
then deprotected by trifluoroacetic acid in
methylene chloride with two equivalents of
triethylsilane to give crude (2a) which was

5   purified by reverse phase HPLC. The biphenyl-
based peptidomimetic (8) was prepared by the
reductive amination of 4-amino-3'-methyl biphenyl
with N-Boc-S-trityl cysteinal, to give the N-Boc-
S-trityl derivatives of (8), which was then

10   deprotected by trifluoroacetic acid and purified
by reverse phase HPLC. The peptidomimetics (4)
and (5) were prepared from the reductive amination
of 4-amino-3'-tert.butoxycarbonyl biphenyl and 4-
amino-4'-tert.butoxycarbonylbiphenyl,

15   respectively, with N-Boc-S-trityl cysteinal, to
give the N-Boc-S-trityl, tert-butyl ester of (4)
and (5). Deprotection by trifluoroacetic acid and
purification by reverse phase HPLC gave pure (4)
and (5).

20   **Synthesis**

        The basic approach used for the preparation
of the compounds of the invention is illustrated
in Scheme 1 with the synthesis of compound 4.
[Compound numbers in the following discussion

25   refer to Schemes 1 and 2.]

Scheme 1. Representative Synthesis of FTase Inhibitors[a]
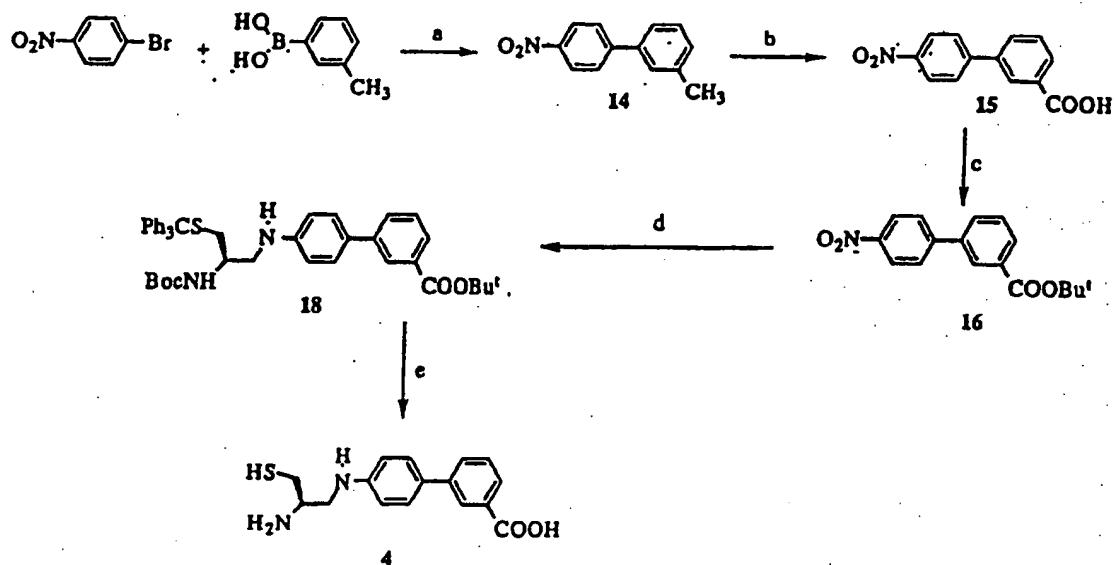
[a] Reagents: (a) Pd(OAc)₂; (b) KMnO₄, pyridine/H₂O; (c) (1) (COCl)₂, (2) *tert*-butyl alcohol, *n*-BuLi; (d) (1) H₂, Pd/C, (2) *N*-Boc-*S*-tritylcysteinal 17, (3) NaB(CN)H₃; (e) TFA, Et₃SiH.

Scheme 2. Synthesis of Compounds 11 and 12[a]
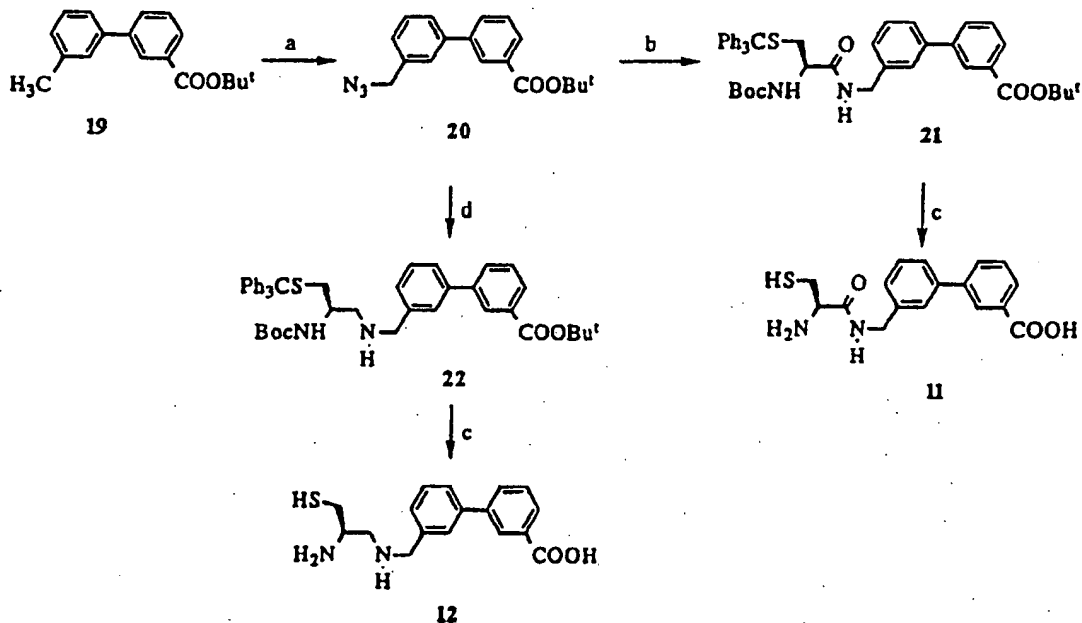
1-Bromo-4-nitrobenzene was coupled to 3-
methylphenylboronic acid (34) through an modified
Suzuki coupling (30) to afford compound **14** (35).
Compound **14** was oxidized to carboxylic acid **15**

5   which was converted to the acid chloride and
reacted with lithium tert-butoxide (36) to give
the tert-butyl ester **16**. Reduction of **16** by
hydrogenation and subseqent reductive amination
(37) of the resulting amine with N-Boc-S-trityl-

10   cysteinal **17** (38) gave the fully protected
derivative **18**, which was deprotected by
trifluoroacetic acid in the presence of
triethylsilane (39). Compound **4** was purified by
reverse phase HPLC and isolated as its

15   trifluoroacetate salt by lyophilization.
        The synthesis of **11** and **12** is described in
Scheme 2. Compound **19** was made from 3-methyl-3'-
carboxybiphenyl (itself formed from aryl-aryl
coupling of methyl 3-bromobenzoate with 3-

20   methylphenylboronic acid followed by a
saponification) via the same method as compound
16. Bromination of **19** followed by reaction with
sodium azide gave **20** which as catalytically
hydrogenated to give the corresponding amine.

25   Reaction of Boc-trityl protected cysteine with
this amine through the mixed anhydride method gave
21, while compound **22** was made by reductive
amination with Boc-trityl protected cysteinal.

Experimental

30   ^{1}H and ^{13}C NMR spectrum were recorded on a
Bruker AM-300 spectrometer. Chemical shifts were
reported in δ (ppm) relative to tetramethylsilane.
All coupling constants were described in Hz.
Elemental analyses were performed by Atlantic

35   Microlab Inc., Georgia. Optical rotations were
measured on a Perkin-Elmer 241 polarimeter.

Concentrations are expressed in g/mL. Flash
column chromatography was performed on silica gel
(40-63 μm) under a pressure about 4 psi. Solvents
were obtained from commercial suppliers and
5   purified as following: tetrahydrofuran and ether
were distilled from sodium benzophenone ketyl,
methylene chloride was distilled over lithium
aluminum hydride. Preparative HPLC was performed
using a Waters 600 E controller and a Waters 490 E
10   Multi-Wavelength UV detector with a 25x10 cm
Delta-Pak C-18 300 Å cartridge column inside a
Waters 25x10 cm Radial Compression Module.
Analytical HPLC was performed using a Rainin HP
Controller and a Rainin UV-C detector with a
15   Rainin 250x4.6 mm 5 μm Microsorb C-18 column.
High resolution mass spectra (HRMS) and low
resolution mass spectra (LRMS) were performed on a
Varian MAT CH-5 and VG 7070 mass spectrometer.
The purity of all the synthesized inhibitors was
20   more than 98% as indicated by analytical HPLC.

A. 4-Nitro-3'-methylbiphenyl (14).

To a mixture of 4-nitrobenzene (3.0 g, 14.8
mmol) and 3-methylphenylboronic acid (2.06 g, 15.1
mmol) in 35 mL of acetone and 40 mL of water was
25   added $K_2CO_3 \cdot 1.5H_2O$ (5.93 g, 37.5 mmol) and $Pd(OAc)_2$
(101 mg, 0.50 mmol). The deep black mixture was
refluxed for 6 hr and then cooled. The mixture
was extracted with ether and the organic layer was
passed through a layer of celite. The pale yellow
30   solution was dried over Na_2SO_4 and evaporated to
dryness. The residue was recrystallized from hot
methanol to give pale yellow crystals (2.68 g,
85%). m.p.59-60 °C.^{1}H NMR ($CDCl_3$) δ 8.26 (d, 8.7
Hz, 2H), 7.70 (d, 8.7 Hz, 2H), 7.41 (m, 3H), 7.26
35   (d, 7.1Hz, 1H), 2.43 (s, 1H). ^{13}C NMR ($CDCl_3$) δ
147.6, 146.8, 138.8, 138.6, 129.6, 128.9, 128.0,

127.6, 124.4, 123.9, 21.4. LRMS (EI) for
$C_{13}H_{11}NO_2$213 (M⁺, intensity 100); HRMS (EI) calcd
213.0789, obsd 213.0778.

B.   4-Nitro-3'-carboxybiphenyl (15).

5   Compound 14 (2.31 g, 10 mmol) was suspended
in a mixture of 10mL of pyridine and 20 mL of
water. The mixture was heated to refluxing and
then $KMnO_4$ (7.9 g, 50 mmol) was added in portions.
This mixture was refluxed for 1 hr and then
10   stirred at room temperature for 4 hr. The hot
mixture was filtered and the black solid was
washed with hot water. The filtrate was acidified
with 6 N HCl. The precipitate was collected and
dried (2.16 g, 89%). m.p. 265°C (decomp). ¹H NMR
15   (DMSO-d_6) δ 11.1-11.4 (br s, COOH), 8.32 (d, 8.7
Hz, 2H), 8.27 (s, 1H), 8.02 (m, 4H), 7.66 (t, 7.8
Hz, 1H). ¹³C NMR (DMSO-d_6) δ 167.1, 148.9, 145.6,
138.2, 131.8, 131.5, 129.6 (br), 127.9, 124.2
(br). LRMS (EI) for $C_{13}H_9O_4N$ 243 (M⁺, 100), 152
20   (60); HRMS (EI) calcd 243.0531, obsd 243.0544.
Anal. ($C_{13}H_9NO_4$) C, H, N.

C.   4-Nitro-3'-tert-butoxycarbonylbiphenyl (16).
To a solution of 15 (1.215 g, 5 mmol) in 30
mL of methylene chloride was added oxalyl chloride
25   (0.65 mL, 7.45 mmol) and one drop of DMF. The
mixture was stirred until no further bubbling was
observed. The clear solution was exaporated to
dryness to give the crude acid chloride. To
another flask containing 7.0 mL of tert-butanol
30   was added n-BuLi (1.8 M in hexane, 2.8 mL, 5.04
mmol) under a water bath. The turbid solution was
stirred for 5 min at room temperature and then the
above acid chloride in 20 mL of THF was added
through a dropping funnel. The mixture was
35   stirred overnight before the solvents were

evaporated.   The residue was dissolved into
methylene chloride and washed with 0.5 N NaOH.
The organic layer was dried over MgSO$_4$ and
evaporated.   The residue was recrystallized from

5   methanol to give pale yellow crystals (851 mg,
57%). m.p. 110.5-111.0°C. ^{1}H NMR (CDCl$_3$) δ 8.32
(d, 7.8 Hz, 2H), 8.24 (s, 1H), 8.06 (d, 7.7 Hz,
1H), 7.77 (m, 3H), 7.56 (t, 7.7 Hz, 1H), 1.63 (s,
9H). ^{13}C NMR (CDCl$_3$) δ 165.1, 147.1, 146.5, 138.7,

10   132.8, 131.0, 129.6, 129.0, 128.2, 127.8, 124.0,
81.4, 28.0, LRMS (EI) for C$_{17}$H$_{17}$O$_4$N 299 (M$^+$, 20), 243
(70), 266 (30), 152 (25; HRMS (EI) calcd 299.1157,
obsd 299.1192. Anal. (C$_{17}$H$_{17}$NO$_4$) C, H, N.

D. N-Boc-S-trityl cysteinal (17).

15   To a solution of N-Boc-S-trityl cysteine
(7.44 g, 16 mmol) in 85 mL of methylene chloride
was added triethylamine (2.22 mL, 16 mmoL) and
N,O-dimethylhydroxylamine hydrochloride (1.57 g,
16.1 mmol). This mixture was cooled in an ice

20   bath and 1-(3-dimethylaminopropyl)-3-
ethylcarbodiimide hydrochloride (EDCI, 3.08 g,
16.0 mmol) and HOBT (2.17 g, 16 mmol) was added.
The mixture was stirred at 0°C for 1 hr and at
room temperature for a further 10 hr. The mixture

25   was extracted with methylene chloride and 0.5 N
HCl. The organic layer was washed consecutively
with 0.5 N HCl, concentrated NaHCO$_3$ and brine. The
organic layer was dried and evaporated. The
residue was purified by flash column

30   chromatography (1.5:1=hexane:ethylacetate) to give
a white foam (7.40 g, 91%).m.p.59-60°(decomp). ^{1}H
NMR (CDCl$_3$) δ 7.41 (m, 6H), 7.20-7.31 (m, 9H), 5.13
(d, 8.9 Hz, 1H), 4.76 (br s, 1H), 3.64 (s, 3H),
3.15 (s, 3H), 2.56 (dd, 4.7 and 12.1 Hz, 1H), 2.39

35   (dd, 7.8 and 12.1 Hz, 1H), 1.43 (s, 9H). ^{13}C NMR
(CDCl$_3$ δ 170.7, 154.9, 144.2, 129.3, 127.6, 126.4,

79.3, 66.4, 61.2, 49.5, 33.8, 31.8, 28.1. This
carboxyamide (2.02 g, 4.0 mmol) was dissolved in
30 mL of ether and cooled to -10°c. Lithium
aluminum hydride (167 mg, 4.40 mmol) was added and
5   the mixture was stirred for 15 min under the
nitrogen. Then 40 mL of 0.5 N HCl was added and
the solution was extracted with ether. The ether
layer was washed with 0.5 N HCl and dried. The
evaporation of solvents gave a white foam (1.80 g)
10 which was used for further reaction without
purification. The ^{1}H NMR spectrum of this compound
was complex. The percentage of the aldehyde was
about 65-70%, which was calculated according to
the integration of the sharp singlet (δ 9.17) and
15 the trityl peak (δ 7.40, m, 6H;7.28,m,9H).
Lowering the temperature to -45°C did not improve
the aldehyde percentage.

E.   4-N-[2(R)-tert-butoxycarbonylamino-3-
triphenylmethylthipropyl]amino-3'-tert-
20 butoxycarbonylibiphenyl (18).
Compound 16 (768 mg, 2.56 mmol) was dissolved
in THF. A catalytic amount of 10% Pd on activated
carbon (78 mg) was added. The mixture was
hydrogenated (40 psi) for 30 min. The black
25 mixture was passed through a thin layer of celite
and the pale yellow solution was evaporated. The
residue was dissolved in 10 mL of methanol. To
this solution was added 0.5 mL of acetic acid and
a solution of the same equivalents of aldehyde 17
30 (according to the ^{1}H NMR determination) in 6 mL of
methanol. Sodium cyanoborohydride (241 mg, 3.84
mmol, 1.5 eq) was added and the mixture was
stirred overnight. After the evaporation of
solvents, the residue was extracted with ethyl
35 acetate and concentrated sodium bicarbonate. The
organic layer was dried and evaporated. The

residue was purified by flash column
chromatography (3.5:1-hexane:THF) to give a white
foam (1.09 g, 61%). m.p. 75.0-76.0 °C(decomp).
$[\alpha]^{25}_D$=-2.13 (c=0.01, $CH_3COOC_2H_5$). ^1H NMR ($CDCl_3$) δ
8.14 (s, 1H), 7.86 (d, 7.7 Hz, 1H), 7.66 (d, 7.8
Hz, 1H), 7.40 (m, 9H), 7.22-7.30 (m, 9H), 6.61 (d,
8.5 Hz, 2H), 4.58 (d, 7.1 Hz, 1H), 3.83 (br m, 2H,
Cys α proton and the amine), 3.12 (br m, 2H, CH_2N),
2.48 (br, m, 2H, CH_2S), 1.60 (s, 9H), 1.44 (s, 9H).
^{13}C NMR ($CDCl_3$) δ 165.9, 155.6, 147.5, 144.4,
141.2, 132.3, 130.1, 129.5, 129.2, 128.5, 128.0,
127.9, 127.1, 126.8, 112.9, 80.9, 79.7, 67.0,
49.4, 47.1, 34.3, 28.3, 28.2 (expect 14 aromatic
C, observed 13). Anal. ($C_{44}H_{48}N_2O_4S \cdot 1.2H_2O$) C, H, N,
S.

F. 4-N-[2(R)-amino-3-mercaptopropyl]amino-3'-
carboxybiphenyl (4).

Compound 18 (600 mg, 0.85 mmol) was dissolved
in 2mL of TFA and 2 mL of methylene chloride.
Triethylsilane was added dropwise to the deep
brown mixture until the brown color had
disappeared. The mixture was then kept at room
temperature for 1 hr. Then solvents were
evaporatee and the residue was dried under vacuum.
The solid was triturated with 30 mL of ether and
3mL of 3 N Hcl in ether. The white precipitate
was filtered and washed with ether to obtain a
crude product (270 mg, 84%). This crude product
was dissolved into 30 mL of dilute Hcl solution
(0.01N) and was lyophilized. Analytical HPLC
showed the purity to be 95%.m.p. 105-106 °C
(decomp). $[\alpha]^{25}_D$=+13.16 (c=0.01 in methanol. ^1J M<R
(CD_3OD δ 8.18 (s, 1H), 7.89 (d, 7.7 Hz, 1H), 7.78
(d, 7.3 Hz, 1H), 7.49 (m, 3H), 6.82 (d, 8.5 Hz,
2H), 3.56 (m, 2H CHN and CH_2N), 3.42 (dd, 8.9 and
15.2 Hz, 1H, CH_2S). ^{13}C NMR (D_2O and CD_3OD) δ

171.1, 147.1, 141.4, 131.9, 130.9, 130.1, 128.8,
128.5, 127.0, 115.2, 53.2, 45.4, 25.0 LRMS (FAB,
glycerol) for $C_{16}H_{18}N_2O_2S$ (M+1) 303. Anal.
($C_{16}H_{18}N_2O_2S \cdot 2HCl$) C, H, N, S. Further purification
5   by preparative HPLC (Waters C-18, 40%
acetonitrile, 60% water, 0.1% TFA, 40 min
gradient) gave product **4** (120 mg) with a purity
over 99.9%.

G.   **3-Methyl-3'-tert-butoxycarbonylbiphenyl (19).**
10   The coupling of 3-methylphenylboronic acid
with 3-bromobenzoic acid methyl ester gave a 3-
methyl-3'-methoxycarbonylbiphenyl (79% yield),
which was then hydrolyzed to yield a 3-methyl-3'-
carboxybiphenyl (97% yield). Compound **19** (an oil)
15   was prepared from this acid using the same method
as for the preparation of compound **16** (65% yield).
^{1}H NMR (CDl$_3$) δ 8.21 (s, 1H), 7.95 (d, 7.8 Hz, 1H),
7.73 (d, 6.6 Hz), 1H), 7.46 (m, 3H), 7.35 (t, 7.5
Hz, 1H), 7.20 (d, 7.4 Hz, 1H), 2.43 (s, 3H), 1.62
20   (s, 9H). ^{13}C NMR (CDCl$_3$) δ 165.6, 141.3, 140.2,
138.3, 132.4, 140.0, 128.7, 128.5, 128.3, 128.0,
127.9, 124.2, 81.0, 28.1, 21.4. LRMS (EI) for
$C_{18}H_{20}O_2$ 268 (M$^+$, 35), 212(100), 195 (20); HRMS (EI)
calcd 268.1463, obsd 268.1458.

25   H.   **3-Azido-3'-tert-butoxycarbonylbiphenyl (20).**
Compound **19** (2.18 g, 8.13 mmol) and N-
bromosuccinimide (1.70 g, 9.50 mmol) was suspended
in 60 mL of CCl$_4$. Dibenzoyl peroxide (20 mg) was
added and the mixture was refluxed for 1.5 hr.
30   After removing the solid, the filtrate was washed
with concentrated sodium bicarbonate and dried
over sodium sulfate. ^{1}H NMR showed the crude
material contained 70% of monobrominated and 30%
of dibrominated product. This material was
35   dissolved in 20 mL of DMSO and sodium azide (3.70

- 104 -

g, 57 mmol) was added. The mixture was heated to
80 °C for 4 hr before being poured into a mixture
of methylene chloride and water. The organic
layer was dried and evaporated. The residue was
5   purified by flash column chromatography (5% of
ethyl acetate in hexane) to give 20 (2.14 g, 78%,
two steps) as colorless oil. ^{1}H NMR (CDCl$_3$) δ 8.22
(s, 1H), 8.00 (d, 7.7 Hz, 1H), 7.76 (d, 8.2 Hz,
1H), 7.58 (m, 2H), 7.50 (m, 2H), 7.33 (d, 7.6 Hz,
10   1H), 4.43 (s, 2H), 1.62 (s, 9H). ^{13}C NMR (CDCl$_3$) δ
165.2, 140.5, 140.3, 135.8, 132.3, 130.7, 129.1,
128.5, 128.2, 127.8, 127.1, 126.7, 126.6, 80.9,
54.3, 27.8.

I. N-Boc-S-trityl-cysteinyl-3-aminomethyl-3'-
15   tert-butoxycarbonylbiphenyl (21).
Compound 20 (0.75 g, 2.43 mmol) was dissolved
in 30 mL of methanol. A catalytic amount of 5%
palladium on barium sulfate (0.30 g) was added.
The mixture was hydrogenated at 1 atm for 5 hr.
20   The catalyst was removed by filtration and the
methanol was evaporated. This residue was
dissolved in 40 mL of methylene chloride. N-Boc-
S-trityl cysteine (1.12 g, 2.43 mmol) was added at
0 °C followed by EDCI (1 eq) and HOBT (1 eq). The
25   mixture was stirred for 24 hr. After workup and
evaporation of solvents, the residue was purified
by flash column chromatography (hexane:ethyl
acetate=3.2:1) to give 21 (570 mg, 44%). m.p. 84-
86 °C. ^{1}H NMR (CDCl$_3$) δ 8.17 (s, 1H), 7.95 (d, 7.7
30   Hz, 1H), 7.70 (d, 7.7 Hz, 1H), 7.50-7.30 (m, 9H),
7.30-7.10 (m, 11H), 6.44 (br, 1H), 4.86 (br, 1H),
4.45 (d, 4.0 Hz, 2H, CH$_2$Ph), 3.87 (br, 1H, Cys α
H), 2.75 (dd, 7.2 and 12.8 Hz, 1H, CH$_2$S), 2.55 (dd,
5.3 and 12.8 Hz, 1H, CH$_2$S), 1.62 (s, 9H), 1.36 (s,
35   9H). Anal. (C$_{45}$H$_{48}$N$_2$O$_5$S) C, H, N, S.

J.   Cysteinyl-3-aminomethyl-3'-carboxybiphenyl
(11).

Compound 21 (150 mg) was deprotected using
the same method as for the preparation of compound
4.   Final purification by preparative HPLC gave 11
as a white solid (42 mg, 46%). m.p. 88-89 °C
(decomp). ^{1}H MNR (CD$_3$OD) δ 8.26 (s, 1H), 8.01 (d,
7.7 Hz, 1H), 7.86 (d, 7.7 Hz, 1H), 7.64 (s, 1H),
7.56 (m, 2H), 7.46 (t, 7.6 Hz, 1H), 7.35 (d, 7.6
Hz, 1H), 4.53 (s, 2H), 4.00 (t, 5.2 Hz, 1H, Cys α
H), 3.06 (dd, 14.6 and 5.2 Hz, 1H, CH$_2$S), 2.97 (dd,
14.6 and 6.8 Hz, 1H, CH$_2$S). LRMS (EI) for
C$_{17}$H$_{18}$N$_2$O$_3$S 331 (M+1, 8), 281 (100), 226 (75), Anal.
(C$_{17}$H$_{18}$N$_2$O$_3$S·HCl·0.6H$_2$O) C, H, N.

K.   3-N-[2(R)-tert-Butoxycarbonylamino-3-
triphenylmethylthiopropyl]aminomethyl-3'-tert-buto
xy-carbonylbiphenyl (22).

The azide 20 (900 mg, 2.91 mmol) was
dissolved in 20 mL of methanol. A catalytic
amount of 5% Pd on barium sulfate (90 mg) was
added.   This mixture was hydrogenated at 1 atm
overnight.   The catalyst was removed and the
methanol was evaporated.   The remaining residue
was dissolved in a mixture of 0.5 N HCl (20 mL)
and ether (20 mL).   The aqueous phase was
neutralized with 1 N NaOH and extracted into
methylene chloride.   After the evaporation of
solvents, a viscous oil was obtained (600 mg,
73%). ^{1}H NMR (CDCl$_3$) δ 8.22 (s, lH), 7.97 (d, 7.8
Hz, 1H), 7.75 (d, 7.7 Hz, 1H), 7.57 (s, 1H), 7.50
(m, 2H), 7.43 (t, 7.7 Hz, 1H), 7.33 (d, 7.4 Hz,
1H), 3.96 (s, 2H), 1.62 (s, 9H), 1.46 (br s, 2H,
NH$_2$). This amine (581 mg, 2.05 mmol) was dissolved
in 10 mL of methanol and 0.5 mL of acetic acid
before N-Boc-S-tritylcysteinal (leq, according to
^{1}H NMR determination of the aldehyde percentage)

was added. Sodium cyanoborohydride (193 mg, 1.50
eq) was added to the above solution and the
mixture was stirred at room temperature overnight.
After workup, the crude residue was purified by
5   flash column chromatography (1: 1 = ethyl acetate:
hexane) to give a white foam (602 mg, 41%). m.p.
66-68 °C (decomp). ^{1}H NMR (CDCl$_3$) δ 8.21 (s, 1H),
7.96 (d, 7.7 Hz, 1H), 7.73 (d, 8.0 Hz, 1H),
7.37-7.51 (m, 10H), 7.15-7.31 (m, 10H), 4.69 (br
10   d, 1H), 3.75 (br s, 3H, PhCH$_2$N and Cys α H), 2.68
(dd, 6.0 and 12.3 Hz, 1H, CH$_2$S), 2.56 (dd, 5.5 and
12.3 Hz, 1H, CH$_2$S), 2.47 (m, 1H, CH$_2$N), 2.35 (m,
1H, CH$_2$N), 1.62 (s, 9H), 1.42 (s, 9H), 1.12 (br s,
1H, NH).

15   **L. 3-N-[2(R)-amino-3-mercaptopropyl]**
aminomethyl-3'-carboxybiphenyl (12).
Compound 22 (480 mg, 0.672 mmol) was
dissolved in a mixture of 2 mL of methylene
chloride and 2 mL of trifluoroacetic acid.
20   Several drops of triethylsilane were added until
the deep brown color had disappeared. This
mixture was kept at room temperature for 1.5 hr,
and then the solvents were evaporated, and the
residue was dried under vacuum. The solid residue
25   was dissolved in 1 mL of acetic acid and 2 mL of
HCl (1.7 M) in acetic acid. Finally 5 mL of HCl
(3 M) in ether and 10 mL of ether were added. The
white precipitate was washed with dry ether and
dried to give a hydrochloride salt (215 mg, 81%).
30   ^{1}H NMR (D$_2$O) δ 8.16 (s, 1H), 7.94 (d, 7.7 Hz, 1H),
7.85 (d, 7.7 Hz, 1H), 7.70 (s, 2H), 7.55 (t, 7.8
Hz, 2H), 7.46 (d, 7.5 Hz, 1H), 4.36 (s, 2H, PhCH$_2$),
3.81 (m, 1H, Cys α H), 3.57 (dd, 5.7 and 13.7 Hz,
1H, CH$_2$N), 3.44 (dd, 6.5 and 13.7 Hz, 1H, CH$_2$N),
35   2.97 (dd, 5.3 and 15.1 Hz, 1H, CH$_2$S), 2.86 (dd, 5.9
and 15.1 Hz, 1H, CH$_2$S).

M.   2-Methoxy-4-nitro-3'-tert-butoxycarbonyl-
biphenyl (23).

The coupling of 1-bromo-2-methoxy-4-
nitrobenzene with 3-methylphenylboronic acid
followed by the oxidation gave the 2-methoxy-4-
nitro-3'-carboxybiphenyl. The reaction of acid
chloride with lithium tert-butoxide gave 23 (3
steps, 35%). m.p. 88.0-88.5 °C. ^{1}H NMR (CDCl$_3$) δ
8.13 (s, 1H), 8.00 (d, 7.7 Hz, 1H), 7.89 (d, 8.3
Hz, 1H), 7.81 (s, 1H), 7.69 (d, 7.7 Hz, 1H), 7.48
(m, 2H), 3.90 (s, 3H), 1.60 (s, 9H). ^{13}C NMR
(CDCl$_3$) δ 165.2, 156.7, 148.0, 136.3, 136.2, 133.2,
132.0, 130.8, 130.1, 129.0, 127.9, 115.8, 106.0,
81.1, 55.9, 27.9. LRMS (EI) for C$_{18}$H$_{19}$NO$_5$ 329 (M$^+$,
30), 273 (100).

N.   2-Methoxy-4-N-[2(R)-N-tert-butoxycarbonyl-
amino-3-triphenylmethylthiopropyl]amino-
3'-tert-butoxycarbonylbiphenyl (24).

Compound 24 was prepared using the same
method as for the preparation of compound 18
(yield 63%). m.p. 76.0-77.0 °C (decomp). [α]^{25}D=-
11.25 (c=0.01, CH$_3$COOC$_2$H$_5$). ^{1}H NMR (CDCl$_3$) δ 8.09
(s, 1H), 7.86 (d, 7.0 Hz, 1H), 7.65 (d, 7.0 Hz,
1H), 7.37 (t, 7.7 Hz, 1H), 7.43 (m, 6H), 7.21-7.32
(m, 9H), 7.11 (d, 8.1 Hz, 1H), 6.21 (s, 1H), 6.18
(d, 8.1 Hz, 1H), 4.58 (d, 6.1 Hz, 1H), 3.86 (br s,
1H), 3.76 (s and m, 4H), 3.14 (br d, 4.9 Hz, 2H),
2.49 (br d, 5.1 Hz, 2H), 1.59 (s, 9H), 1.43 (s,
9H). ^{13}C NMR (CDCl$_3$) δ 165.9, 157.3, 155.5, 148.8,
144.3, 138.9, 133.3, 131.5, 131.2, 130.0, 129.4,
127.8, 127.5, 126.7, 118.7, 104.7, 96.2, 80.5,
79.4, 66.8, 55.2, 49.3, 47.0, 34.1, 28.2, 28.1.
Anal. (C$_{45}$H$_{50}$N$_2$O$_5$S) C, H, N, S.

O.   2-Methoxy-4-N-[2(R)-amino-3-mercaptopropyl]
amino-3'-carboxybiphenyl (10).

Compound **10** was obtained from the
deprotection of compound **24**. m.p. 120-121 °C
(decomp). $[\alpha]^{25}D$=+12.62 (c=0.01, in methanol). ^{1}H
NMR (CD$_3$OD) δ 8.09 (s, 1H), 7.89 (d, 7.8 Hz, 1H),

5   7.67 (d, 7.8 Hz, 1H), 7.43 (t, 7.7 Hz, 1H), 7.20
(d, 8.1 Hz, 1H), 6.56 (s, 1H), 6.53 (d, 8.1 Hz,
1H), 3.81 (s, 3H), 3.60 (m, 2H, Cys α H and CH$_2$N),
3.48 (m, 1H, CH$_2$N), 2.96 (dd, 4.9 and 13.7 Hz, 1H,
CH$_2$S), 2.86 (dd, 5.4 and 13.7 Hz, 1H, CH$_2$S). ^{13}C

10   NMR (D$_2$O and CD$_3$OD) δ 171.1, 158.2, 149.3, 139.7,
135.1, 132.2, 131.1, 130.4, 129.4, 128.4, 120.5,
106.2 (broad, due to deuterium exchange), 98.8,
56.3, 53.4, 45.1, 24.9. LRMS (EI) for C$_{17}$H$_{20}$N$_2$O$_3$S 332
(M$^+$). Anal. (C$_{17}$H$_{20}$N$_2$O$_3$S$\cdot$1.2HCl$\cdot$H$_2$O) C, H, N, S.

15   **P.   Cysteinyl-4-amino-3'-carboxybiphenyl (6).**
Compound 6 was purified through preparative
HPLC. Purity was shown to be over 99%. m.p.
120.0-121.0 °C. ^{1}H NMR (CD$_3$OD) δ 8.25 (s, 1H), 7.98
(d, 7.6 Hz, 1H), 7.84 (d, 7.7 Hz, 1H), 7.74 (d,

20   7.0 Hz, 2H), 7.54 (t, 7.7 Hz, 1H), 7.66 (d, 8.6
Hz, 2H), 4.16 (q, 5.0 Hz, 1H), 3.19 (dd, 5.20 and
14.8 Hz, 1H), 3.07 (dd, 7.7 and 14.7 Hz, 1H); ^{13}C
NMR (CD$_3$OD) δ 169.8, 166.7, 141.9, 138.7, 137.8,
132.6, 132.2, 130.2, 129.5, 128.8, 128.5, 121.6,

25   56.9, 26.4. LRMS (El) for C$_{16}$H$_{16}$O$_3$N$_2$S 316 (M$^+$, 25),
213 (100); HRMS (EI) calcd 316.0882, obsd
316.0867. Anal. (C$_{16}$H$_{16}$N$_2$O$_3$S$\cdot$CF$_3$COOH$\cdot$H$_2$O) C, H, N.

Q.   4-N-[2(R)-Amino-3-mercaptopropyl]amino-2'-
carboxybiphenyl (3).

30   m.p. 129-130 °C (decomp). $[\alpha]^{25}D$=+12.58
(c=0.01, CH$_3$OH). ^{1}H NMR (CD$_3$OD) δ 7.73 (d, 7.6 Hz,
1H), 7.50 (d, 7.6 Hz, 1H), 7.35 (m, 2H), 7.21 (d,
8.5 Hz, 2H), 6.86 (d, 8.5 Hz, 2H), 3.58 (m, 2H),
3.46 (m, 1H), 2.97 (dd, 4.8 and 14.6 Hz, 1H), 2.86

35   (dd, 5.4 and 14.6 Hz, 1H). ^{13}C NMR (D$_2$O and CD$_3$OD) δ

174.3, 146.3, 141.9, 132.8, 132.7, 131.4, 130.6,
130.2, 127.9, 115.3, 52.9, 45.8, 25.0. LRMS (FAB,
glycerol) for $C_{16}H_{18}N_2O_2S$ (M+1) 303. Anal.
($C_{16}H_{18}N_2O_2S \cdot 1.6HCl$) C, H, N, S.

R.   4-N-[2(R)-Amino-3-mercaptopropyl]amino-4'-
carboxybiphenyl (5).

     m.p. 260 °C (decomp). $[\alpha]^{25}D=+12.20$ (c=0.01,
CH_3OH). ^1H NMR (CD_3OD) δ 8.03 (d, 8.5 Hz, 2H), 7.66
(d, 8.4 Hz, 2H), 7.56 (d, 8.4 Hz, 2H), 6.85 (d,
8.5 Hz, 2H), 3.57 (m, 2H), 3.45 (m, 1H), 2.98 (dd,
4.8 and 14.5 Hz, 1H), 2.85 (dd, 5.7 and 14.5 Hz,
1H). ^{13}C NMR (D_2O and CD_3OO) δ 169.8, 146.4, 146.1,
133.6, 131.4, 129.8, 129.4, 127.1, 117.0, 53.3,
47.2, 25.5. LRMS (EI) for $C_{16}H_{18}O_2N_2S$ 302 (M$^+$, 15),
285 (15), 226 (100), 213 (50). HRMS (EI) calcd
302.1088, obsd 302.1089. Anal. ($C_{16}H_{18}N_2O_2S \cdot 2HCl$) C,
H, N.

S.   4-N-[2(R)Amino-3-Mercaptopropyl]aminobiphenyl
(7).

     m.p. 216 °C (decomp). $[\alpha]^{25}D=+13.27$ (c=0.01,
CH_3OH). ^1H NMR (CD_3OD) α 7.54 (m, 4H), 7.39 (m,
2H), 7.26 (m, 1H), 6.82 (br s, 2H), 3.56 (br m,
2H), 3.45 (m, 1H), 2.98 (m, 1H), 2.87 (m, 1H); ^{13}C
NMR (CD_3OD) δ 144.8, 141.8, 135.7, 129.9, 129.1,
127.8, 127.4, 117.4, 53.2, 47.6, 25.5. LRMS (EI)
for $C_{15}H_{18}N_2S$ 258 (M+, 15), 182 (100); HRMS (EI)
calcd 258.1190, obsd 258.1183. Anal.
($C_{15}H_{18}N_2S \cdot 1.6HCl$) C, H, N, S.

T.   4-N-[2(R)-Amino-3-mercaptopropyl]amino-
3'-methylbiphenyl (8).

     ^1H NMR (CD_3OD) δ 7.50 (d, 8.2 Hz, 2H), 7.35
(m, 2H), 7.27 (t, 7.6 Hz, 1H), 7.08 (d, 7.3 Hz,
1H), 6.95 (d, 8.2 Hz, 2H), 3.60 (m, 2H), 3.46 (m,
1H), 2.99 (dd, 4.9 and 14.6 Hz, 1H), 2.88 (dd, 5.5

and 14.6 Hz, 1H), 2.37 (s, 3H). LRMS (EI) for
$C_{16}H_{20}N_2S$ 272 (M+, 15), 196 (100). HRMS (EI) calcd
272.1341, obsd 272.1347.

U.   4-N-[2(R)-Amino-3-mercaptopropyl]amino-3'-
5   methoxycarbonylbiphenyl (9).

m.p. 86-89 °C (decomp). ^{1}H NMR (CD$_3$OD) δ 8.18
(s, 1H), 7.90 (d, 7.7 Hz, 1H), 7.79 (d, 6.6 Hz,
1H), 7.55 (d, 8.6 Hz, 2H), 7.49 (t, 7.7 Hz, 1H),
7.01 (d, 8.6 Hz, 2H), 3.92 (s, 3H), 3.543.65 (m,
10   2H), 3.44-3.52 (m, 1H), 2.97 (dd, 4.7 and 14.7 Hz,
1H), 2.88 (dd, 5.5 and 14.7 Hz, 1H). LRMS (EI) for
$C_{17}H_{20}O_2N_2S$ 316 (M+, 15), 299 (20), 240 (100). HRMS
(EI) calcd 316.1240, obsd 316.1239. Anal.
($C_{17}H_{20}N_2O_2S \cdot 2HCl$) C, H, N, S.

Table 2

Table of Microanalysis Data

Compd	Formulae	C% (cal, obs)	H% (cal, obs)	N% (cal, obs)	S% (cal, obs)
3	$C_{16}H_{18}N_2O_2S \cdot 1.6HCl$	53.27 (53.41)	5.44 (5.78)	7.77 (7.35)	8.87 (8.47)
4	$C_{16}H_{18}N_2O_2S \cdot 2HCl$	51.20 (51.60)	5.37 (5.30)	7.47 (7.07)	8.53 (8.22)
5	$C_{16}H_{18}N_2O_2S \cdot 2HCl$	51.20 (51.62)	5.37 (5.56)	7.47 (7.00)	
6	$C_{16}H_{16}N_2O_3S \cdot CF_3COOH \cdot H_2O$	48.21 (48.24)	4.24 (4.20)	6.25 (6.32)	
7	$C_{15}H_{18}N_2S \cdot 1.6HCl$	56.89 (57.04)	6.19 (6.46)	8.85 (8.74)	10.11 (10.03)
9	$C_{17}H_{20}N_2O_2S \cdot 2HCl$	52.44 (52.84)	5.65 (5.92)	7.19 (7.37)	8.22 (8.53)
10	$C_{17}H_{20}N_2O_3S \cdot 1.2HCl \cdot H_2O$	51.80 (51.91)	5.89 (5.96)	7.11 (6.81)	8.12 (7.77)
11	$C_{17}H_{18}N_2O_3S \cdot HCl \cdot 0.6H_2O$	54.07 (54.11)	5.35 (5.39)	7.42 (7.35)	
15	$C_{13}H_9NO_4$	64.19 (64.05)	3.70 (3.75)	5.76 (5.80)	
16	$C_{17}H_{17}NO_4$	68.23 (68.07)	5.68 (5.73)	4.68 (4.64)	
18	$C_{44}H_{48}N_2O_4S \cdot 1.2H_2O$	73.17 (72.82)	6.98 (6.83)	3.88 (3.87)	4.43 (4.50)
21	$C_{45}H_{48}N_2O_5S$	74.14 (73.74)	6.64 (6.74)	3.84 (3.79)	4.39 (4.32)
24	$C_{45}H_{50}N_2O_5S$	73.97 (73.72)	6.85 (7.04)	3.83 (3.66)	4.38 (4.32)

Table 3
Examples of Peptidomimetics of the Invention

	Compound		Structure
	2	FTI-232	Cys-4-aminobenzoyl-Met
5	2a	FTI-249	red.Cys-4-aminobenzoyl-Met
	3	FTI-273	red.Cys-4-amino-2'-carboxybiphenyl
	4	FTI-265	red.Cys-4-amino-3'-carboxybiphenyl
	5	FTI-271	red.Cys-4-amino-4'-carboxybiphenyl
	6	FTI-278	Cys-4-amino-3'-carboxybiphenyl
10	7	FTI-268	red.Cys-4-aminobiphenyl
	8	FTI-263	red.Cys-4-amino-3'-methylbiphenyl
	9	FTI-259	red.Cys-4-amino-3'-carboxymethylbiphenyl
	10	FTI-281	red.Cys-4-amino-2-OMe-3'-carboxybiphenyl
	11	FTI-285	red.Cys-4-amino-2-phenyl-3'-methylbiphenyl
15	12	FTI-238	Cys-3-aminomethyl-3'-carboxybiphenyl
		FTI-283	red.Cys-3-aminomethyl-3'-carboxybiphenyl
		FTI-282	(DL)4-(2,3-diaminopropyl)-amino-3' carboxybiphenyl
		FTI-288	red.Cys-4-amino-2-OPr-3'-carboxybiphenyl
		FTI-289	red.Cys-4-amino-2-phenyl-3'-carboxybiphenyl
20		FTI-291	4-(3-aminoalanyl)-amino-3'-carboxybiphenyl
		FTI-292	(L)4(2,3-diaminopropyl)-amino-3'-carboxybiphenyl
		FTI-295	4-(Ethylsulfonyl-3-aminoalanyl)-amino-3'-carboxy-biphenyl
25		FTI-296	4-(Vinylsulfonyl-3-aminoalanyl)-amino-3'-carboxybiphenyl
		FTI-2102	red.Cys-4-amino-3'-tetrazolylbiphenyl

Number designations used for compounds of the
invention discussed below is shown in Table 3.

EXAMPLE 22

FTase and GGTase I Activity Assay

5   Human Burkitt lymphoma (Daudi) cells (ATCC,
Rockville, MD) were grown in suspension in RPMI
1640 medium containing 10% fetal bovine serum
(FBS) and 1% Pen-Strep in a humidified 10% CO_2
incubator at 37°C. The cells were harvested and
10   sonicated in 50 mM Tris, pH 7.5, 1 mM EDTA, 1 mM
EGTA, 25 μg/ml leupeptin, 1 mM
phenylmethylsulfonyl fluoride. Homogenates were
then spun at 12,000 x g and the resulting
supernatant further spun at 60,000 x g. The
15   supernatant was assayed for both FTase and GGTase
I. Briefly, 100 μg of the supernatants was
incubated in 50 mM Tris, pH 7.5, 50 μM $ZnCl_2$, 20 mM
KCl, 3 mM $MgCl_2$ and 1 mM DTT. For FTase assays,
the reaction was incubated at 37°C for 30 minutes
20   with recombinant H-Ras-CVLS (11 μM) and [³H] FPP
(625 nM; 16.3 Ci/mmol). For GGTase assays, the
reaction was also incubated for 30 minutes at 37°C
but with recombinant H-Ras-CVLL (5 μM) and [³H]
GGPP (525 nM; 19.0 Ci/mmol). The reaction was
25   stopped and passed through glass fiber filters to
separate free and incorporated label. For
inhibition studies, the peptidomimetics were
premixed with FTase or GGTase I prior to adding
the remainder of the reaction mixture.
30   Recombinant H-Ras-CVLS was prepared as described
previously (26) from bacteria (31). Recombinant
H-Ras-CVLL was prepared from bacteria (32).

EXAMPLE 23

Peptidomimetics Farnesylation Assay

The ability of human Burkitt lymphoma (Daudi)
FTase to farnesylate peptides and peptidomimetics
was determined as described previously (34, 35).
Briefly, 25 μl of reaction mixture containing 50
μg of 60,000 xg supernatants and 20 μM
peptidomimetic in 50 mM Tris, pH 7.5, 50 μM $ZnCl_2$,
20 mM KCl, 3 mM $MgCl_2$, 1 mM DTT and 0.2% octylß-D-
glucoside was incubated for 30 minutes at 37°C,
then spotted onto silica gel G TLC sheets (20 x 20
cm, Brinkmann Instruments), and developed with n-
propanol/5 N ammonium hydroxide/water (6:1:1).
The dried sheets were sprayed with En^3Hance (DuPont
NEN) and exposed to x-ray film for detection of
$[^3H]$ farnesylated products.

EXAMPLE 24

Ras and Rap1A Processing Assay

EJ3 cells were treated with peptidomimetics
or vehicle for 20-24 h. Cells were lysed in lysis
buffer (10 mM Na_2HPO_4, pH 7.25, 150 mM NaCl, 0.1%
sodium dodecyl sulfate, 1% Triton X-100, 12 mM
sodium deoxycholate, 1 mM NaF, 0.2% NaN_3, 2 mM
PMSF, 25 μg/ml leupeptin) and the lysates were
cleared by spinning at 13,000 rpm for 15 minutes.
Ras protein was immunoprecipitated overnight at
4°C with 50 μg of anti-Ras antibody (Y13-259;
hybridoma from ATCC, Rockville, MD) along with 30
μl Protein A-agarose goat anti-rat IgG complex
(Oncogene Science, Uniondale, NY).
Immunoprecipitates were washed 4 times with lysis
buffer and the bound proteins were released by
heating for 5 minutes in 40 μl SDS-PAGE sample
buffer and subsequently electrophoresed on a 12.5%
SDS-PAGE. Proteins were transferred onto
nitrocellulose and subsequently blocked with 5%

non-fat dry milk in PBS (containing 1% Tween 20
(PBS-T) and probed with Y13-259 (50 μg/ml in 3%
non-fat dry milk in PBS-T). Positive antibody
reactions were visualized using peroxidase-

5   conjugated goat anti-rat IgG (Oncogene Science,
Uniondale, NY) and an enhanced chemiluminescence
detection system (ECL; Amersham).

For Rap1A processing assays, 50 μg of cell
lysates were electrophoresed as described above

10   for Ras processing and transferred to
nitrocellulose. These membranes were then blocked
with 5% milk in Tris-buffered saline, pH 8.0,
containing 0.5% Tween-20 and probed with anti-
Rap1A (1 μg/ml in 5% milk/TBS-T; Santa Cruz

15   Biotechnology, Santa Cruz, CA). Antibody
reactions were visualized using peroxidase-
conjugated goat anti-rabbit IgG (Oncogene) and ECL
chemiluminescence as described above.

Structural Modeling (Figure 13)

20   The calculation of the energy minimized
conformations was carried out using the AMBER
force field within the MacroModel program, version
3.5a.

EXAMPLE 25

25   The potency of the peptidomimetics of Figure
12 and Table 3 for inhibiting partially purified
FTase was evaluated by determining their ability
to inhibit the transfer of farnesyl to recombinant
H-Ras as described above. The results are

30   summarized in Table 4, which indicates the IC_{50}s
obtained for FTase activity and GGTase-I activity,
and the selectivity for a number of
peptidomimetics of the invention. The IC_{50} values
given in Table 4 represent inhibition of FTase and

35   GGTase I in vitro by the listed compounds.

Table 4

In vitro Activity of CAAX Mimetic Inhibitors of FTase

Inhibitor	FTase IC$_{50}$ (nM)	GGTase-I IC$_{50}$ (nM)	Selectivity	Substrate
FTI-232	150	1500	10	
FTI-249	300	4400	15	
FTI-273	543 (3)[a]	140,000 (2)[a]	258	nd[b]
FTI-265	114 (10)	100,000 (6)	877	no
FTI-271	4575 (4)	>100,000 (2)	>22	no
FTI-278	13,500 (2)	100,000 (2)	7	nd
FTI-268	1,070 (3)	>100,000 (3)	93	no
FTI-263	710 (3)	>100,000 (3)	141	no
FTI-259	917 (3)	>100,000 (3)	109	no
FTI-281	40 (6)	43,600 (5)	1090	nd
FTI-238	100,000 (2)	>100,000 (3)	>1	no
FTI-283	11,000 (1)	35,000 (2)	3	nd
FTI-285	2075 (4)	8500 (2)	4	
FTI-282	50,000 (1)	>>100,000 (1)	>2	
FTI-288	41 (6)	2375 (4)	59	
FTI-289	16 (5)	643 (4)	40	
FTI-291	210,000 (1)		0	
FTI-292	60,000 (1)		0	
FTI-295	200,000 (1)	>1,000,000 (1)	>5	
FTI-296	430,000 (1)	>>1,000,000 (1)	>2	
FTI-2102	30 (1)	5,000 (1)	187	

[a]Numbers in parentheses indicate number of determinations.
Where no number is given, at least two determinations were
made.

[b]nd indicates not determined.

The results obtained showed that compound 2,
i.e. Cys-4ABA-Met (1-10 μM) inhibited FTase in a
concentration-dependent manner with an IC_{50} of 150
nM (FTI-232, Table 4). This value is similar to
5   the previously reported IC_{50} values for CVIM and
Cys-4ABA-Met (35). Reduction of the amide bond
between cysteine and aminobenzoic acid gave the
red-Cys-4ABA-Met (2a, FTI-249) which had an IC_{50} of
300 nM. However, replacing the methionine and the
10   C-terminal amide bond in (2a) by another aromatic
ring to obtain the biphenyl-based peptidomimetic
(4) improved potency by twofold (FTI-265, Table
4). Peptidomimetic 4 had an IC_{50} of 114 nM towards
partially purified FTase from human Burkitt
15   lymphoma cells and 50 nM towards rat brain FTase
purified to homogeneity. Thus, despite major
structural differences between the compound CVIM
(1) and 4, the latter (4) retained the potent
FTase inhibitory activity of the tetrapeptide CVIM
20   (1) and the peptide mimetics 2 and 2a.

As noted, Figures 14A and 14B graphically
illustrate the results of FTase and GGTase I
inhibition studies. In these studies, partially
purified FTase and GGTase I were incubated with
25   the peptidomimetics to be tested and their ability
to transfer [^{3}H] farnesyl to H-Ras-CVLS (FTase) and
[^{3}H] geranylgeranyl to H-Ras CVLL (CCTase I) was
determined as described. Figure 14A shows FTase
inhibition by: □, (4) and ■, (5) while Figure 14B
30   plots FTase (□) and GGTase I (■) inhibition by
(4). Each curve is representative of at least
four independent experiments.

Geranylgeranylation is a more common protein
prenylation than farnesylation (49). It is,
35   therefore, advantageous for CAAX peptidomimetics
targeting farnesylation to have high selectivity
towards inhibiting FTase compared to GGTase. In

the CAAX tetrapeptides, the X position determines
whether the cysteine thiol will be farnesylated by
FTase or geranylgeranylated by GGTase I. Those
proteins or peptides with Leu or Ile at the X
5   position are geranylgeranylated. As shown in
Table 4, the present compounds do not
significantly inhibit GGTase I and demonstrate
much greater selectivity for FTase.

Figure 14B shows that compound 4, which is a
10   potent FTase inhibitor, is a very poor GGTase I
inhibitor. The ability of compound 4 to inhibit
the transfer of geranylgeranyl to Ras-CVLL (IC_{50} =
100,000 nM) was found to be 877-fold less than
that of 4 to inhibit the transfer of farnesyl to
15   Ras-CVLS (IC_{50} = 114 nM) (Table 4). This
selectivity was much more pronounced than in the
peptidomimetics 2 and 2a which were more selective
for FTase relative to GGTase I by only 10 and 15-
fold, respectively. It is also noted that the
20   free carboxylate of compound 4 is not responsible
for this selectivity since replacement of this
group by a methyl in compound 8 did not increase
affinity towards GGTase I (Table 4). These
results indicate that the FTase and GGTase I
25   binding sites are quite different and that
differences between Leu, Ile and Met side chains
cannot be the only predictors of selectivity.
Regardless of the explanation, it is clear that
the compounds of the invention are much more
30   selective to inhibition of FTase.

Besides having poor cellular uptake and being
rapidly degraded, another disadvantage of natural
CAAX peptides is that they are farnesylated by
FTase. This results in metabolic inactivation
35   since farnesylated CAAX derivatives are no longer
inhibitors of FTase (34). Figure 15 shows that
the natural peptide CVLS (carboxyl terminal CAAX

of H-Ras) is farnesylated by FTase from Burkitt
lymphoma cells. Replacing the tripeptide VLS with
4-amino-3'-hydroxycarbonylbiphenyl, as in 4 did
not affect potency towards FTase inhibition (Table

5   4) but prevented farnesylation of the cysteine
thiol (Figure 15). None of the peptidomimetics of
the invention is metabolically-inactivated by
FTase (Figure 15). Thus, although AAX tripeptides
are not necessary for potent FTase inhibition,

10   they appear to be required for farnesylation.

        With reference to Figure 15, it is to be
noted that the transfer of [^{3}H] farnesyl to
peptides and peptidomimetic by FTase was
determined by silica G TLC as described below.

15   FPP, F-peptide, and ORIGIN designate farnesyl
pyrophosphate, farnesylated peptide and origin,
respectively. Figure 15 shows: Lane 1, FPP only;
lane 2, FPP and CVLS but no FTase; lane 3, FPP and
FTase but not peptide. Lanes 4-9 all contained

20   FTase and FPP with lane 4, CVIM; lane 5, CVLS;
lane 6, compound 2a; lane 7, compound 4; lane 8,
compound 5; lane 9, compound 8. The results shown
indicate that the compounds of the invention are
not farnesylated in contrast to the CAAX

25   compounds. Data given are representative of two
independent experiments.

        The foregoing results show that the novel
peptidomimetics described herein have two very
important features, namely, they are potent FTase

30   inhibitors, and they are resistant to metabolic
inactivation by FTase. Another important feature
is that the present compounds inhibit Ras
processing in whole cells. This is shown by the
following with reference to Figure 16 which

35   illustrates Ras and Rap1A processing. To this
end, Ras transformed 3T3 cells were treated with
inhibitors, lysed and the lysate A)

- 120 -

immunoprecipitated with anti-Ras antibody or B)
separated by SDS-PAGE. Immunoprecipitates from A)
were separated by SDS-PAGE and blotted with anti-
Ras antibody whereas samples from B) were blotted

5   with anti-Rap1A antibody as described hereafter.
Figure 16 shows: Lane 1, control; lane 2,
lovastatin; lane 3, reduced **2a** (200 μM); lane 4, **4**
(100 μM); lane 5, **4** (50 μM); lane 6, **4** (25 μM);
lane 7, **5**; lane 8, **8**. Data are representative of

10   3 independent experiments. Farnesylated Ras runs
faster than unprocessed Ras on SDS-PAGE (23-25,
28, 29). Figure 16A (lane 1) shows that cells
treated with vehicle contain only processed Ras
whereas cells treated with lovastatin (lane 2)

15   contained both processed and unprocessed Ras
indicating that lovastatin inhibited Ras
processing. Lovastatin, an HMG-CoA reductase
inhibitor which inhibits the biosynthesis of
farnesylpyrophosphate and

20   geranylgeranylpyrophosphate, is used routinely as
a positive control for inhibition of processing of
both geranylgeranylated and farnesylated proteins
(36, 37, 39, 40, 51). Cells treated with reduced
Cys-4ABA-Met **3** in its free carboxylate forms did

25   not inhibit Ras processing. However, in contrast,
the corresponding methyl ester of **2a** (200 μM)
inhibited FTase (Figure 16A, lane 3). This is
consistent with previous work that showed that
neutralization of the carboxylate of CAAX peptides

30   enhances their ability to inhibit Ras processing
(37, 40, 51). Although compound **4** has a free
carboxylate negative charge, it was able to enter
cells and potently inhibit Ras processing (lane 4,
100 μM compound **4**). It was found that compound **4**

35   inhibited Ras processing with concentrations as
low as 50 μM (lane 5), whereas its corresponding
parent compound **2a** did not inhibit Ras processing

at concentrations as high as 200 μM. Compound 4
was as potent as the methylester of its parent
compound (2a) (Figure 16A, lane 3). Furthermore,
4 appears to be the first CAAX peptidomimetic that
5   effectively inhibits Ras processing in whole cells
directly without relying on cellular enzymes for
activation. The hydrophobic character of the
biphenyl group apparently compensates for the free
carboxylate negative charge thus allowing the
10   peptidomimetic to penetrate membranes and
promoting its cellular uptake.

The selectivity of the present Ras
farnesylation inhibitors has also been
investigated by determining their ability to
15   inhibit processing of Rap1A, a small G-protein
that is geranylgeranylated (49, 50). Cells were
treated with lovastatin or peptidomimetics exactly
as described for Ras processing experiments.
Lysates were then separated by SDS-PAGE and
20   immunoblotted with anti-Rap1A antibody as
described below. Control cells contained only the
geranylgeranylated Rap1A (Figure 16B, lane 1)
whereas lovastatin-treated cells contained both
processed and unprocessed forms of Rap1A
25   indicating, as expected, that lovastatin inhibited
the processing of Rap1A (Figure 16B, lane 2).
Compound 4, which inhibited Ras processing, was
not able to inhibit Rap1A geranylgeranylation
(Figure 16B, lanes 4-6). Compounds 5 and 8 also
30   did not inhibit Rap1A processing (Figure 16B,
lanes 7 and 8).

Structures for a number of compounds of the
invention are summarized in Table 3, and their
relative effectiveness in inhibiting FTTase and
35   GGTase shown in Table 4. The activity of the
inhibitors is reported in Table 4 as IC_{50} values,
the concentration at which FTase or GGTase I

activity was inhibited by 50%. Some of the
inhibitors were further characterized for their
ability to serve as substrates for farneylsation
by thin layer chromatography, as shown in Table 4

5   (41).

        The published sequence dependence studies on
FTase have shown a strong preference for
methionine in the terminal position of CAAX. In
the compounds of the present invention, no

10   methionine residue is present and the tripeptide
AAX is completely replaced by a simple hydrophobic
moiety. The most potent inhibitor in the CAAX
series is Cys-Ile-Phe-Met (18, 22) with an IC_{50}
value of 30 nM. Peptidomimetic inhibitor 10 is as

15   potent as CIFM despite the large difference
between their structures. These results confirm
the hydrophobic strategy for AAX replacement
according to the invention.

        As previously reported, the incorporation of

20   an aromatic amino acid into the A_2 position of
CA_1A_2X (such as CIFM) prevents the tetrapeptide
from serving as a substrate for farneyslation
(22). Table 4 shows that the designed non-peptide
CAAX mimetics (such as compound 4) are not

25   substrates for farnesylation. This lack of
farnesylation by FTase may be due to the inhibitor
binding to the enzyme in a conformation that does
not permit farnesyl transfer to the thiol group.

        III. Geranylgeranyl tranferase Inhibitors

30   The carboxyl terminal CAAX tetrapeptide of
Ras is a substrate for FTase and serves as a
target for designing inhibitors of this enzyme
with potential anticancer activity (33). Our
earlier application describes a highly potent

35   (IC_{50}=500 pM) inhibitor of FTase, FTI-276 (Fig. 17)

(66). Its cell-permeable methyl ester FTI-277
inhibits H-Ras processing in whole cells with an
IC_{50} of 100 nM (66). Furthermore, FTI-276 is
highly selective (100-fold) for FTase over GGTase
5   I (Table 5).

<p align="center">TABLE 5</p>

	In Vitro (IC_{50}, nM)			In Vivo Processing (IC_{50}, uM)		
	FTase	GGTase I		H-Ras	K-Ras	Rap1A
PTI-276	0.5	50	PTI-277	0.1	10	50
GGTI-287	25	5	GGTI-286	>30	2	2
GGTI-297	270	40	GGTI-298	>20	3	3

<p align="center">EXAMPLE 26</p>

Synthesis of FTase and GGTase I Inhibitors

15   Peptidomimetics FTI-276 and FTI-277 were
prepared as described above. GGTase I inhibitors
GGTI-287 and GGTI-286 were prepared from 2-phenyl-
4-nitrobenzoic acid (66) by reaction with L-
leucine methyl ester followed by reduction with
20   stannous chloride. The resulting 4-amino-2-
phenylbenzoyl leucine methyl ester was reacted
with N-Boc-S-trityl-cysteinal and deprotected by
procedures similar to those described for the
FTase inhibitors (66) to give GGTI-286 and GGTI-
25   287 as their hydrochloride salts.

**A.   4-Amino-2-phenylbenzoyl-(S)-methionine
        methyl ester hydrochloride**

        To a mixture of 70 mL of acetone and 85 mL of
water was added 2-bromo-4-nitrotoluene 6.84 g (30
30   mmol), phenylboronic acid 3.84 g (31.5 mmol),
potassium carbonate 10.35 g (75 mmol) and
palladium acetate 336 mg (1.5 mmol). The mixture
was refluxed for 10 hr and then extracted with
ether and dilute hydrochloric acid. After
35   evaporating solvents, the solid residue was
recrystallized from methanol to give 5.64 g of 4-

<p align="center">- 124 -</p>

nitro-2-phenyltoluene (88% yield). ^{1}H NMR (CDCl$_3$) δ
8.09-8.11 (m, 2H), 7.40-7.49 (m, 4H), 7.30-7.33
(m, 2H), 2.37 (s, 3H).

5   The above 4-nitro-2-phenyltoluene (4.46 g, 21
mmol) was suspended in 21 mL of pyridine and 42 mL
of water. The mixture was heated to boiling
followed by addition of potassium permanganate
(19.8, 126 mmol). The mixture was refluxed for 2
hr and then filtered to remove the solids. The
10   filtrate was acidified with 6N HCl to give 4.48 g
of 4-nitro-2-phenylbenzoic acid (89% yield). ^{1}H
NMR (CDCl$_3$) δ 8.25-8.33 (m, 2HO, 8.08 (d, 8.9 Hz,
1H), 7.41-7.51 (m, 3H), 7.31-7.39 (m, 2H).

       The above 4-nitro-2-phenylbenzoic acid (2.43
15   g, 10 mmol) was suspended in 50 mL of methylene
chloride. To this solution was added (L)-
methionine methyl ester hydrochloride (2.0 g,
10 mmol), triethylamine (1.38 mL, 10 mmol), 1-(3-
dimethylaminopropyl)-3-ethylcarbodiimide
20   hydrochloride (EDCI, 2.01 g, 10.5 mmol), 1-
hydroxybenzotriazole (HOBT, 1.35 g, 10 mmol). The
mixture was stirred for 12 hr and then extracted
with methylene chloride and 1N hydrochloric acid.
After the evaporation of solvents, the residue was
25   recrystallized from ethyl acetate and hexane to
give 3.22 g of 4-nitro-2-phenylbenzolyl-(L)-
methionine methyl ester (yield 83%). ^{1}H NMR
(CDCl$_3$) δ 8.24-8.28 (m, 2H), 7.85 (d, 8.9 Hz, 1H),
7.43-7.52 (m, 5H), 6.01 (d, 7.5 Hz, 1H), 4.69
30   (ddd, 1H), 3.68 (s, 3H), 2.05 (m, 2H), 1.98 (s,
3H), 1.88-1.96 (m, 1H), 1.72-1.81 (m, 1H).

       The 4-nitro-2-phenylbenzoyl-(L)-methionine
methyl ester (3.04 g, 7.83 mmol) was dissolved
into 100 mL of ethyl acetate followed by the
35   addition of stannous chloride hydrate (8.84 g, 39
mmol). The mixture was refluxed for 2 hr and then
extracted with a mixture of ethyl acetate and

concentrated sodium bicarbonate. After the
evaporation of solvents, the residue was dissolved
in methylene chloride followed by addition of 3N
hydrogen chloride in ether. The solid was
5   filtered and dried to give 2.96 g of 4-amino-2-
phenylbenzoyl-(L)-methionine methyl ester
hydrochloride (yield 96%). ^{1}H NMR (CD$_3$OD) δ 7.65
(d, 8.1 Hz, 1H), 739-7.46 (m, 7H), 4.53 (dd, 4.3
and 9.5 Hz, 1H), 3.69 (s, 3H), 2.15-2.23 (m, 1H),
10   2.00 (s, 3H), 1.93-2.11 (m, 2H), 1.74-1.83 (m,
1H); ^{13}C NMR (CD$_3$OD) δ 173.4, 171.7, 143.4, 140.1,
137.4, 134.0, 130.9, 129.7, 129.4, 125.5, 122.7,
53.0, 52.9, 31.3, 30.9, 15.1.

15   **B.   4-[2(R)-tert-butoxycarbonylamino-3-
      triphenylmethylthiopropyl]amino-2-
      phenylbenzoyl-(S)-methionine methyl
      ester**

      To a mixture of 4-amino-2-phenylbenzoyl-(S)-
methionine methyl ester hydrochloride (1.27 g,
20   3.22 mmol) in 20 mL of methanol was added N-Boc-S-
trityl-(L)-cysteinal (1.0 eq, according to ^{1}H NMR
determination of aldehyde percentage) and sodium
cyanoborohydride (400 mg, 2.0 eq). The mixture
was stirred for 12 hr. After the evaporation of
25   solvents, the residue was extracted with ethyl
acetate and concentrated sodium bicarbonate.
After removing solvents, the residue was purified
through flash column chromatography (1:1 = hexane:
ethyl acetate, silica) to give the product 1.67 g
30   (yield 67%). ^{1}H NMR (CDCl$_3$) δ 7.65 (d, 8.6 Hz,
1H), 7.34-7.42 (m, 11H), 7.18-7.29 (m, 9H), 6.52
(dd, 2.3 and 8.1 Hz, 1H), 6.34 (d, 2.3 Hz, 1H),
5.65 (d, 7.7 Hz, 1H), 4.64 (ddd, 1H), 4.55 (d, 8.1
Hz, 1H), 4.19 (br t, 1H), 3.78 (br m, 1H), 3.64
35   (s, 3H), 3.09 (t, 6.1 Hz, 2H), 2.44 (m, 2H), 2.04-
2.10 (m, 2H), 2.00 (s, 3H), 1.81-1.90 (m, 1H),
1.60-1.70 (m, 1H), 1.41 (s, 9H); ^{13}C NMR (CDCl$_3$) δ

172.0, 168.3, 155.7, 149.4, 144.3, 141.6, 141.1,
131.3, 129.5, 128.7, 128.5, 127.9, 127.7, 126.8,
122.6, 113.6, 111.3, 79.8, 67.1, 52.2, 51.7, 49.5,
47.2, 34.3, 31.6, 29.4, 28.2, 15.2.

5   C.   4-[2(R)-amino-3-mercaptopropyl]amino-
             2-phenylbenzoyl-(S)-methionine methyl
             ester hydrochloride

       The above N-Boc-S-trityl protected peptide
10   methyl ester (900 mg) was dissolved in 5 mL of
       methanol. To this mixture was added a solution of
       mercuric chloride (774 mg, 2.50 eq) in 5 mL of
       methanol. The mixture was refluxed for 20 min.
       The precipitate was collected and dried. This
15   solid was suspended in 10 mL of methanol and
       reacted with gaseous hydrogen sulfide. After the
       removal of black solid, the clear solution was
       evaporated to dryness. The residue was then
       dissolved in methylene chloride followed by
20   addition of 3N hydrogen chloride in ether. The
       white solid was collected and dried to give the
       pure product 476 mg (yield 81%). ^{1}H NMR (CD$_3$OD) δ
       7.42 (d, 8.4 Hz, 1H), 7.30-7.38 (m, 5H), 7.77 (d,
       8.4 Hz, 1H), 6.71 (s, 1H), 4.48 (dd, 4.2 and 5.1
25   Hz, 1H), 3.68 (s, 3H), 3.44-3.58 (m, 3H),
       2.90-2.95 (dd, 4.1 and 14.5 Hz, 1H), 2.79-2.85
       (dd, 4.7 and 14.5 Hz, 1H), 2.18-2.22 (m, 1H),
       2.03-2.16 (m, 1H), 2.00 (s, 3H), 1.91-1.97 (m,
       1H), 1.73-1.82-(m, 1H); ^{13}C NMR (CD$_3$OD) δ 173.7,
30   173.4, 150.7, 143.5, 142.3, 131.2, 129.8, 129.5,
       128.6, 125.6, 115.6, 112.2, 53.7, 53.2, 52.8,
       45.0, 31.4, 30.9, 25.3, 15.0.

       D.   4-[2(R)-amino-3-mercaptopropyl]amino-
             2-phenylbenzoyl-(S)-methionine

35   The N-Boc-S-trityl protected peptide methyl
       ester (500 mg) was hydrolyzed with 2.0 eq of
       lithium hydroxide at 0°C for 1 hr. The product

was deprotected with trifluoroacetic acid (2 mL) in methylene chloride (1 mL). Triethylsilane was added dropwise until the deep yellow color disappeared. The mixture was kept at r.t. for 1.5 hr. After the evaporation of solvents, the residue was dried and washed with dry ether. The solid was purified through preparative HPLC to give a pure product 270 mg (yield 78%). ^{1}H NMR (CD$_3$OD) δ 7.44 (d, 8.4 Hz, 1H), 7.30-7.39 (m, 5H), 6.75 (d, 8.4 Hz, 1H), 6.67 (s, 1H), 4.45 (dd, 4.2 and 5.1 Hz, 1H), 3.42-3.58 (m, 3H), 2.90 (dd, 4.3 and 14.5 Hz, 1H), 2.81 (dd, 5.5 and 14.5 Hz, 1H), 2.17-2.23 (m, 1H), 2.09-2.15 (m, 1H), 2.00 (s, 3H), 1.90-1.99 (m, 1H), 1.71-1.81 (m, 1H); ^{13}C NMR (CD$_3$OD) δ 176.4, 173.5, 150.4, 143.0, 141.5, 131.0, 129.7, 129.4, 128.9, 124.6, 115.0, 112.3, 53.3, 49.6, 44.4, 30.8, 30.1, 24.9, 14.8.

E.   4-Nitro-2-phenylbenzoyl-
      (S)-leucine methyl ester

This compound was prepared through the coupling of 4-nitro-2-phenylbenzoic acid with (L)-leucine methyl ester hydrochloride as for the preparation of the methionine derivative (see Example 26, section A). ^{1}H NMR (CDCl$_3$) δ 8.24-8.26 (m, 2H), 7.86 (d, 8.7 Hz, 1H), 7.41-7.46 (m, 5H), 5.71 (d, 7.4 Hz, 1H), 4.57 (ddd, 1H), 3.67 (s, 3H), 1.37-1.46 (m, 1H), 1.08-1.25 (m, 2H), 0.78 (dd, 6H).

F.   4-[2(R)-tert-butoxycarbonyl-
      3-triphenylmethylthiopropyl]amino-2-
      phenylbenzoyl-(S)-leucine methyl ester

This compound was prepared using the same method as for the preparation of methionine derivative (See Example 26, section B), using 4-amino-2-phenylbenzoyl-(S)-leucine methyl ester and N-Boc-S-trityl-(L)-cysteinal as starting

materials. ^{1}H NMR (CDCl$_3$) δ 7.68 (d, 8.6 Hz, 1H),
7.33-7.41 (m, 11H), 7.17-7.29 (m, 9H), 6.50 (d,
8.6 Hz, 1H), 6.31 (s, 1H), 5.43 (d, 7.8 Hz, 1H),
4.60 (d, 6.1 Hz, 1H), 4.47 (ddd, 1H), 4.19 (br t,
5   1H), 3.77 (br m, 1H), 3.62 (s, 3H), 3.09 (t, 5.9
Hz, 2H), 2.45 (br m, 2H), 1.40 (s, 9H), 1.27-1.33
(m, 1H), 1.03-1.18 (m, 2H), 0.75 (dd, 6H); ^{13}C
(CDCl$_3$) δ 173.2, 168.2, 155.6, 149.4, 144.4, 141.7,
141.2, 131.4, 129.5, 128.8, 128.5, 127.9, 127.6,
10   126.8, 122.7, 113.6, 111.3, 79.6, 67.1, 51.9,
50.9, 49.5, 47.1, 41.2, 34.3, 28.3, 24.4, 22.7,
21.8.

G.   4-[2(R)-amino-3-mercaptopropyl]amino-
2-phenylbenzoyl-(S)-leucine methyl ester
15   hydrochloride

This compound was prepared with the same
method as for the preparation of methionine
derivative (see Example 26, section C), using 4-
[2(R)-tert-butoxycarbonyl-3-
20   triphenylmethylthiopropyl]amino-2-phenylbenzoyl-
(S)-leucine methyl ester and mercuric chloride. ^{1}H
NMR (CD$_3$OD) δ 7.42 (d, 8.5 Hz, 1H), 7.31-7.38 (m,
5H), 6.76 (d, 8.5 Hz, 1H), 6.68 (s, 1H), 4.33 (t,
7.8 Hz, 1H), 3.67 (s, 3H), 3.46-3.55 (m, 3H), 2.95
25   (dd, 4.4 and 14.5 Hz, 1H), 2.81 (dd, 5.1 and 14.5
Hz, 1H), 1.44 (t, 7.6 Hz, 2H), 1.18-1.25 (m, 1H),
0.76-0.83 (dd, 4.1 and 6.6 Hz, 6H).

H.   4-[2(R)-amino-3-mercaptopropyl]amino-
2-phenylbenzoyl-(S)-leucine

30   This compound was prepared with the same
method as for the preparation of the methionine
derivative (see Example 26, section D), using 4-
[2(R)-amino-3-mercaptopropyl]amino-2-
phenylbenzoyl-(S)-leucine methyl ester
35   hydrochloride and lithium hydroxide. ^{1}H NMR
(CD$_3$OD) δ 7.42 (d, 8.5 Hz, 1H), 7.29-7.38 (m, 5H),

6.73 (d, 8.5 Hz, 1H), 6.66 (s, 1H), 4.32 (dd, 3.3
and 5.9 Hz, 1H), 3.41-3.57 (m, 3H), 2.94 (dd, 4.3
and 14.5 Hz, 1H), 2.78 (5.2 and 14.5 Hz, 1H), 1.45
(t, 6.7 Hz, 2H), 1.17-1.26 (m, 1H), 0.78-0.83 (t,
5   8.5 Hz, 6H).

I.   **4-Nitro-2-naphthylbenzoic acid**

The coupling of 4-nitro-2-bromobenzoic acid
methyl ester (1.92 g, 7.4 mmol) with 1-
naphthylboronic acid (2.53 g, 14.7 mmol) in the
10   presence of anhydrous sodium phosphate (3.64 g,
22.2 mmol) and palladium
tetrakistriphenylphosphine (426 mg, 0.368 mmol) in
50 mL of DMF at 100°C gave the
4-nitro-2-naphthylbenzoic acid methyl ester (1.66
15   g, 73% yield). ^{1}H NMR (CDCl$_3$) δ 8.34 (d, 8.5 Hz,
1H), 8.28 (s, 1H), 8.14 (d, 8.5 Hz, 1H), 7.92 (d,
8.2 Hz, 2H), 7.47-7.56 (m, 2H), 7.41 (d, 3.8 Hz,
2H), 7.34 (d, 6.8 Hz, 1H), 3.40 (s, 3H). After
the hydrolysis of methyl ester, 1.44 g of product
20   was collected (yield 91%). ^{1}H NMR (CDCl$_3$) δ 8.33
(d, 8.6 Hz, 1H), 8.23 (s, 1H), 8.17 (d, 8.6 Hz,
1H), 7.88 (d, 8.2 Hz, 2H), 7.46-7.52 (m, 2H),
7.38-7.42 (m, 2H), 7.33 (d, 7.0 Hz, 1H); ^{13}C NMR
(CD$_3$COCD$_3$) δ 167.2, 150.1, 143.1, 139.0, 138.2,
25   134.4, 132.5, 132.1, 129.2, 127.3, 126.7, 125.8,
125.9, 123.3.

J.   **4-Nitro-2-naphthylbenzoyl-
      (S)-methionine methyl ester**

The coupling of 4-nitro-2-naphthylbenzoic
30   acid with (L)-methionine methyl ester in the
presence of EDCI and HOBT provided the desired
product (yield 95%). TLC of the product showed
single spot, but ^{1}H NMR showed the presence of
diastereomers caused by the restricted rotation
35   between naphthyl and phenyl rings. ^{1}H NMR (CDCl$_3$)
δ 8.33-8.38 (m, 1H), 8.26 (ss, 1H), 8.14 (d, 8.5

- 130 -

Hz, 0.5H), 8.00 (d, 8.5 Hz, 0.5H), 7.94-7.98 (m,
2H), 7.42-7.65 (m, 5H), 5.98 (t, 1H), 4.42 (m,
1H), 3.56 (s, 1.5H), 3.51 (s, 1.5H), 1.83 (s,
1.5H), 1.74 (s, 1.5H), 1.56-1.64 (m, 1H),
5   1.33-1.45 (m, 2H), 1.09-1.14 (m, 1H).

K.   4-[2(R)-tert-butoxycarbonyl-
        3-triphenylmethylthiopropyl]amino-
        2-naphthyl-(S)-methionine methyl ester

        The reduction of
10   4-nitro-2-naphthylbenzoyl-(L)-methionine methyl
     ester gave a quantitative yield of the amino
     derivative which was reacted with N-Boc-S-trityl
     cysteinal in the presence of sodium
     cyanoborohydride.   After flash column
15   chromatography (1:1 = ethyl acetate : hexane)
     purification, a desired product was obtained
     (yield 40%). TLC showed single spot, but ¹H NMR
     showed diastereomers caused by the restricted
     rotation between the naphthyl and phenyl rings. ¹H
20   NMR (CDCl₃) δ 7.84-7.96 (m, 3H), 7.49-7.66 (m, 4H),
     7.37-7.43 (m, 7H), 7.14-7.27 (m, 9H), 6.60-6.63
     (d, 8.6 Hz, 1H), 6.33 (m, 1H), 5.67 (d, 7.8 Hz,
     0.6H), 5.60 (d, 7.8 Hz, 0.4H), 4.56 (br d, 6.2 Hz,
     1H), 4.35-4.44 (m, 1H), 4.30 (br, 1H), 3.78 (br m,
25   1H), 3.55 (s, 1.9H), 3.38 (s, 1.1H), 3.06 (t, 5.8
     Hz, 2H), 2.44 (m, 2H), 1.90 (s, 1H), 1.79 (s, 2H),
     1.57-1.68 (m, 0.5H), 1.36-1.45 (m, 10H), 1.23-1.32
     (m, 2H), 0.94-0.98 (m, 0.7H).

L.   4-[2(R)-amino-3-mercaptopropyl]amino-
30   2-naphthylbenzoyl-(S)-methionine

        This compound was prepared from the
     N-Boc-S-trityl protected form (section K) by
     saponification followed with acidic cleavage by
     trifluoroacetic acid. The pure compound was
35   obtained through preparative HPLC. ¹H NMR showed
     complicated diastereomers caused by the restricted

rotation of aryl-aryl bond. ^{1}H NMR (CD$_3$OD) δ
7.86-7.94 (m, 2H), 7.73 (d, 8.6 Hz, 0.6H),
7.35-7.67 (m, 5.4H), 6.83-6.88 (m, 1H), 6.63-6.67
(m, 1H), 4.17-4.23 (m, 1H), 3.41-3.58 (m, 3H),
5   2.91 (dd, 4.2 and 14.5 Hz, 1H), 2.80 (dd, 5.3 and
14.5 Hz, 1H), 1.82 (s, 1.4H), 1.80 (s, 1.6H),
1.65-1.77 (m, 1H), 1.41-1.52 (m, 2H), 1.09-1.32
(m, 1H).

M.   **4-Nitro-2-naphthylbenzoyl-**
10   **(S)-leucine methyl ester**

This compound was prepared with the same
method as for the preparation of the methionine
derivative (section J) using 4-nitro-2-
naphthylbenzoic acid, (S)-leucine methyl ester,
15   EDCI and HOBT. ^{1}H NMR (CDCl$_3$) δ 8.34-8.39 (m, 1H),
8.25 (s, 1H), 8.18 (d, 8.6 Hz, 0.6H), 8.02 (d, 8.6
Hz, 0.4H), 7.91-8.00 (m, 2H), 7.62 (t, 7.0 Hz,
0.6H), 7.48-7.58 (m, 3H), 7.41 (t, 7.0 Hz, 1.4H),
5.71 (d, 7.9 Hz, 0.6H), 5.60 (d, 7.9 Hz, 0.4H),
20   4.29 (m, 1H), 3.57 (s, 1.7H), 3.52 (s, 1.3H),
1.05-1.11 (m, 0.5H), 0.88-0.97 (m, 0.7H),
0.69-0.78 (m, 0.5H), 0.41-0.59 (m, 7.0H),
0.19-0.26 (m, 0.6H).

N.   **4-[2(R)-tert-butoxycarbonylamino-3-**
25   **triphenylmethylthiopropyl]amino-2-**
naphthylbenzoyl-(S)-leucine methyl ester

This compound was prepared with the same
method as for the preparation of the methionine
derivative (section K). ^{1}H NMR (CDCl$_3$) δ 7.85-8.00
30   (m, 3H), 7.47-7.67 (m, 4H), 7.39-7.43 (m, 7H),
7.14-7.37 (m, 9H), 6.61 (d, 8.6 Hz, 1H), 6.32 (s,
1H), 5.46 (d, 7.6 Hz, 0.6H), 5.36 (d, 7.6 Hz,
0.4H), 4.55 (d, 7.2 Hz, 1H), 4.20-4.27 (m, 2H),
3.76 (br, 1H), 3.56 (s, 2H), 3.38 (s, 1H), 3.06
35   (t, 5.9 Hz, 2H), 2.43 (m, 2H), 1.36-1.43 (m, 9H),

0.81-1.03 (m, 1H), 0.55-0.67 (m, 2.8H), 0.36-0.45
(m, 4.7H), 0.00-0.09 (m, 0.6H).

O.   4-[2(R)amino-3-mercaptopropyl]amino-
      2-naphthylbenzoyl-(S)-leucine methyl
      ester

This compound was prepared from the
N-Boc-S-trityl methyl ester of the corresponding
compound, using the method of section L. ^{1}H NMR
(CDCl$_3$) δ 7.98 (d, 8.5 Hz, 0.6H), 7.84-7.90
(m, 2.4H), 7.65 (d, 8.5 Hz, 0.4H), 7.43-7.58 (m,
3.6H), 7.34-7.39 (m, 1H), 6.72 (m, 1H), 6.45 (ss,
1H), 5.46 (d, 7.8 Hz, 0.6H), 5.40 (d, 7.7 Hz,
0.4H), 4.64 (m, 1H), 4.23 (m, 1H), 3.54 (s, 2H),
3.30 (s, 1H), 3.25 (m, 1H), 2.97-3.06 (m, 2H),
2.67 (dd, 3.7 and 13.1 Hz, 1H), 2.47 (dd, 6.5 and
13.2 Hz, 1H), 1.45-1.65 (br s, 2H), 0.81-1.03 (m,
1.2H), 0.54-0.67 (m, 3H), 0.36-0.39 (m, 4.3H),
0.00-0.10 (m, 0.7H).

EXAMPLE 27

FTase and GGTase I Activity Assay

FTase and GGTase I activities from 60,000 x g
supernatants of human Burkitt lymphoma (Daudi)
cells (ATCC, Rockville, MD) were assayed exactly
as described previously for FTase (41).
Inhibition studies were performed by determining
the ability of Ras CAAX peptidomimetics to inhibit
the transfer of [^{3}H]-farnesyl and [^{3}H]-
geranylgeranyl from [^{3}H]FPP and [^{3}H]GGPP to H-ras-
CVLS and H-Ras-CVLL, respectively (41).

EXAMPLE 28

Ras and RapIA Processing Assay

H-Ras cells (45) and K-Ras4B Cells (32) were
kind gifts from Dr. Channing Der and Dr. Adrienne
Cox (University of North Carolina, Chapel Hill).

Means of obtaining these cell lines will be easily
recognized by the skilled practitioner.
Cells were seeded on day 0 in 100 mm dishes in
Dulbecco's modified Eagles medium supplemented

5   with 10% calf serum and 1% penicillin-
streptomycin. On days 1 and 2, cells were refed
with medium containing various concentrations of
FTI-277, GGTI-286 or vehicle (10 mM DTT in DMSO).
On day 3, cells were washed and lysed in lysis

10   buffer containing 50 mM HEPES, pH 7.5, 10 mM NaCl,
1% TX-100, 10% glycerol, 5 mM MgCl$_2$, 1 mM EGTA, 25
μg/ml leupeptin, 2 mM PMSF, 2 mM Na$_3$VO$_4$, 1 mg/ml
soybean trypsin inhibitor, 10 μg/ml aprotinin, 6.4
mg/ml Sigma-104® phosphatase substrate. Lysates

15   were cleared (14,000 rpm, 4°C, 15 min) and equal
amounts of protein were separated on a 12.5% SDS-
PAGE, transferred to nitrocellulose, and
immunoblotted using an anti-Ras antibody (Y13-259,
ATCC) or an anti-RapIA antibody (SC-65, Santa Cruz

20   Biotechnology, Santa Cruz, CA). Antibody
reactions were visualized using either peroxidase-
conjugated goat anti-rat IgG (for Y13-259), or
peroxidase-conjugated goat anti-rabbit IgG (for
RapIA) and an enhanced chemiluminescence detection

25   (ECL, Amersham Corp.), as described previously
(41).

EXAMPLE 29
MAP Kinase Immunoblotting
        Cells were treated with FTI-277, GGTI-286, or

30   vehicle and lysed as previously described for Ras
and RapIA processing. Equal amounts of protein
were separated on a 15% SDS-PAGE, transferred to
nitrocellulose, and immunoblotted using an anti-
MAP kinase antibody (erk2, monoclonal, UBI, Lake

35   Placid, NY). Antibody reactions were visualized
using peroxidase-conjugated donkey anti-mouse IgG

- 134 -

(Jackson ImmunoResearch Laboratories Inc., West
Grove, PA) and an enhanced chemiluminescence
detection system (ECL, Amersham Corp.)

EXAMPLE 30

5   *Inhibition of GGTase I by GGTI-286*

GGTI-287 potently inhibited GGTase I *in vitro*
(IC_{50}=5 nM) and was selective towards inhibiting
GGTase I over FTase (IC_{50}=25 nM) (Table 5). Thus,
the substitution of methionine in FTI-276 by a
10   leucine in GGTI-287 (Fig. 17) increased the
potency towards GGTase I by approximately 10-fold
(Table 5). More importantly, it reversed the
selectivity from a FTase to a GGTase I-specific
inhibitor by a factor of 500 (Table 5). To
15   determine whether this selectivity is respected in
whole cells, the cell-permeable methyl ester
derivative of GGTI-287, GGTI-286 (Fig. 17), was
synthesized and used to treat NIH 3T3 cells which
overexpress oncogenic H-Ras-CVLS (31). Cell
20   lysates were electrophoresed on SDS-PAGE and
immunoblotted with an anti-Ras antibody as
described in Example 28. Figure 18 shows that
accumulation of unprocessed H-Ras did not occur at
concentrations lower than 30 μM GGTI-286.
25   Therefore, GGTI-286 is not a good inhibitor of H-
Ras processing in whole cells. However, GGTI-286
was a very potent inhibitor of the processing of
the geranylgeranylated Rap1A protein (IC_{50}=2 μM)
(Fig. 18). Thus, GGTI-286 is more than 15-fold
30   selective for inhibition of geranylgeranylation
over farnesylation processing (Table 5). This
data is in direct contrast to the FTase specific
inhibitor FTI-277 which inhibited H-Ras and RapIA
processing with IC_{50}s of 100 nM and 50 μM,
35   respectively (Fig. 18). Thus, GGTI-286 is 25-fold

more potent than FTI-277 at inhibiting
geranylgeranylation in whole cells (Table 5).

EXAMPLE 31

Inhibition of GGTase I by GGTI-297 and GGTI-298

5   To determine the effect of replacing the
phenyl substituent with a naphthyl on GGTase I
inhibition, 4-[2(R)-amino-3-mercaptopropyl] amino-
2-naphthyl benzoyl-(L)-leucine (GGTI-297) and its
methylester (GGTI-298) were tested. GGTI-297
10   inhibited GGTase I in vitro with an IC_{50} of 40 nM
and was selective towards inhibiting GGTase I over
FTase (IC_{50} = 270 nM) (Fig. 21, Table 5). Thus, the
substitution of phenyl in GGTI-287 by naphthyl in
GGTI-297 decreased the potency towards GGTase I by
15   8 fold and towards FTase by over 10-fold.
However, more importantly, the selectivity for
GGTase I over FTase increased from 5-fold (GGTI-
287) to 7-fold (GGTI-297), as shown in Table 5.
This selectivity was also respected in vivo since
20   concentrations as high as 20 μM did not inhibit H-
Ras processing whereas Rap1A and Ras4B were
completely blocked at 10 μM GGTI-298 (Table 5).

EXAMPLE 32

Inhibition of K-Ras4B Function by GGTI-286

25   The ability of GGTI-286 to inhibit the
processing and signaling of oncogenic K-Ras4B was
then evaluated. NIH 3T3 cells which overexpress
oncogenic K-Ras4B (32) were treated either GGTI-
286 (0-30 μM) or FTI-277 (0-30 μM) and the lysates
30   were immunoblotted with an anti-Ras antibody as
described under Example 28. Figure 19 shows that
GGTI-286 inhibited potently the processing of K-
Ras4B with an IC_{50} of 2 μM. The ability of GGTI-
286 to inhibit the processing of K-Ras4B was much
35   closer to its ability to inhibit the processing of

geranylgeranylated Rap1A ($IC_{50}=2$ μM) than that of
farnesylated H-Ras ($IC_{50}>30$ μM)(Fig. 18) (Table 5).
This suggested that K-Ras4B might be
geranylgeranylated. Consistent with this is the
5   fact that K-Ras4B processing was very resistant to
the FTase-specific inhibitor FT-277 ($IC_{50}=10$ μM)
(Fig. 19). Furthermore, GGTI-286 inhibited K-
Ras4B processing at concentrations (1-3 μM) (Fig.
19) that had no effect on the processing of
10   farnesylated H-Ras (Fig. 18).

                        EXAMPLE 33
            Effects of GGTI-286 on Oncogenic K-Ras 4B
            Constitutive Activation of MAP Kinase

               To determine whether inhibition of K-Ras4B
15   processing by GGTI-286 results in disruption of
oncogenic signaling, the ability of GGTI-286 to
antagonize oncogenic K-Ras 4B constitutive
activation of MAP kinase was examined. Activated
MAP kinase is hyperphosphorylated and migrates
20   slower than hypophosphorylated (inactive) MAP
kinase on SDS-PAGE (43, 66). Figure 20 shows that
K-Ras4B transformed cells contained mainly
activated MAP kinase. Treatment of these cells
with the FTase-specific inhibitor FTI-277 (0-30
25   μM) did not inhibit MAP kinase activation until 30
μM (Fig. 20). In contrast, GGTI-286 inhibited MAP
kinase activation with an IC_{50} of 1 μM and the
block was complete at 10 μM. Thus, GGTI-286
blocked oncogenic K-Ras4B MAP kinase activation at
30   a concentration (10 μM) where FTI-277 had no
effect. In contrast, oncogenic H-Ras activation
of MAP kinase was inhibited only slightly by GGTI-
286 whereas FTI-277 completely blocked this
activation at 3 μM (Fig. 20). Furthermore, GGTI-
35   286 blocked K-Ras4B activation of MAP kinase at a

                          - 137 -

concentration (10 μM) that had little effect on H-Ras activation of MAP kinase (Fig. 20).

EXAMPLE 34

Antitumor Efficacy

5   The above examples demonstrate that GGTI-286 is a potent and highly selective inhibitor of K-Ras4B processing and activation of oncogenic signalling. In order to demonstrate the efficacy of these inhibitors as anticancer agents, K-Ras4B

10   transformed NIH-3T3 cells were implanted subcutaneously in nude mice.   When the tumors reached sizes of 50-100 mm³, the mice were randomly separated into control and treated groups (5 animals per group, each animal had a tumor on both

15   the right and the left flank). Figure 22 shows that tumors from control animals treated with saline once daily grew to an average size of 2900 mm³ over a period of two weeks. In contrast, tumors from animals treated once daily with GGTI-

20   286 (25 mg/kg or 50 mg/kg) grew to a size of 1600 mm³ or 900 mm³, respectively (Fig. 22). Thus, GGTI-286 inhibited tumor growth by 50% and 70%, respectively.

       In summary, the data clearly identifies GGTI-

25   286 not only as a potent antagonist of K-Ras4B oncogenic signaling in cultured cells, but also as an inhibitor of tumor growth in whole animals.

       References cited herein are listed below for convenience and are hereby incorporated by

30   reference.

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5   It will be appreciated that various

modifications may be made in the invention as

described above without departing from the scope

and intent of the invention as defined in the

10   following claims wherein:

CLAIMS

WE CLAIM:

    1.   A peptidomimetic of the formula:

$$C\beta X$$

5   wherein

    C   is a cysteinyl moiety, in a reduced or nonreduced state;

    X   is an amino acid; and

    β is a residue of an aminobenzoic acid or

10   an aminonaphthoic acid.

    2.   A peptidomimetic according to claim 1 wherein the cysteinyl moiety is in the reduced state.

    3.   A peptidomimetic according to claim 2

15   wherein β is 2-phenyl-4-aminobenzoic acid.

    4.   A peptidomimetic according to claim 1 of the formula:
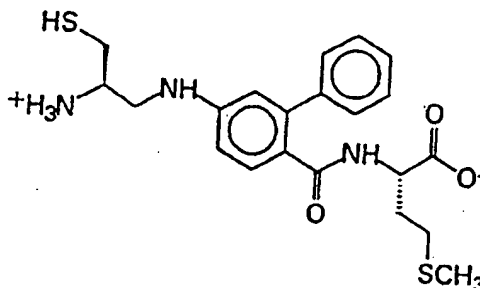
    5.   A peptidomimetic according to claim 1

20   wherein β is a substituted 4-aminobenzoic acid.

    6.   A peptidomimetic according to claim 1 wherein X is methionine or phenylalanine.

7.   A pharmaceutical composition comprising
a peptidomimetic according to claim 1 and a
pharmaceutically acceptable carrier.

8.   A method of inhibiting
5   farnesyltransferase in a host where the
farnesyltransferase is present which comprises
administering to the host an effective amount of a
peptidomimetic according to claim 1.

9.   A peptidomimetic according to claim 1 in
10   the form of a pro-drug.

10.   A pro-drug according to claim 9 wherein
the pro-drug comprises a compound as defined with
one or more terminal amino, sulfhydryl and acid
groups functionalized with a lipophilic, esterase-
15   sensitive moiety.

11.   A pro-drug according to claim 9 wherein
the terminal amino and sulfhydryl groups of the
cysteine C are functionalized by benzyloxy
carbonyl groups or other cleavable lipophilic
20   groups and a terminal carboxylic acid group is
esterified.

12.   A pro-drug according to claim 11 wherein
the carboxylic acid group is esterified as the
methyl ester.

13. A compound of the formula:

wherein R represents H, CH$_3$, or any
lipophilic esterase-sensitive moiety,
and R^1 represents H or a substituted or
5   unsubstituted phenyl group.

14. A compound according to claim 13 wherein
R^1 is an unsubstituted phenyl group, or an alkoxy-,
chloro-, bromo- or methyl- substituted phenyl
group.

10   15. A compound according to claim 13 wherein
R^1 is chosen from the group consisting of a 3,5
dimethylphenyl radical, a thiophene radical, a
naphthyl radical, a pyrrole radical, a pyridyl
radical, an alkyl radical, and an alkoxy radical.

15   16. A compound of the formula
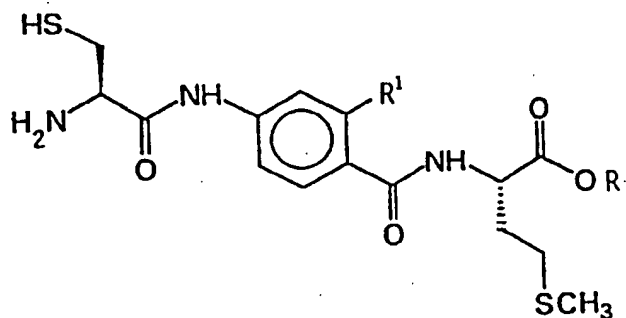
wherein R represents H, CH₃ or any lipophilic esterase-sensitive moiety,

and R¹ represents H or a substituted or unsubstituted phenyl group.

5   17.   A compound according to claim 15 wherein R¹ is an unsubstituted phenyl group, or an alkoxy-, chloro-, bromo- or methyl- substituted phenyl group.

           18.   A compound according to claim 16 wherein
10   R¹ is a 3,5 dimethylphenyl radical.

           19.   A compound of the formula
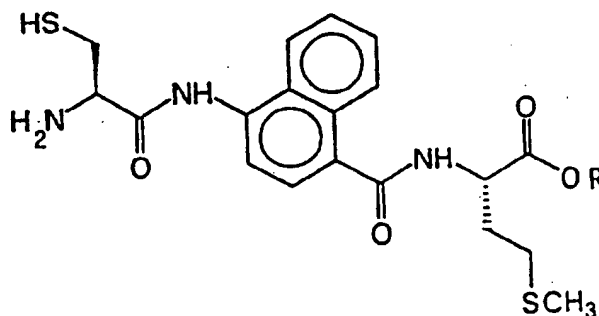
     wherein R represents H, CH₃ or any lipophilic esterase-sensitive moiety.

     20.   A compound of the formula
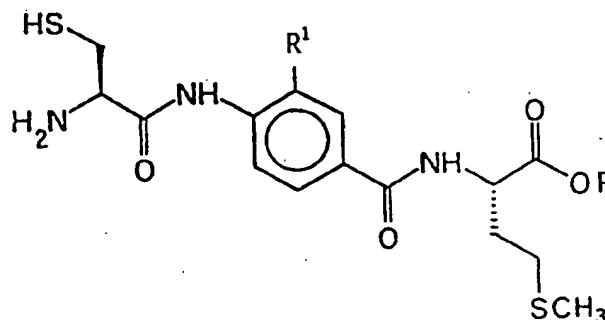
wherein R represents H, CH₃ or any lipophilic
esterase-sensitive moiety,
        and R¹ represents H, CH₃ or OCH₃.

        21.   A method of inhibiting
5 farnesyltransferase in a host wherein the
farnesyltransferase is present which comprises
administering to the host an effective amount of a
peptidomimetic according to claim 13.

        22.   A method of treating cancer comprising
10 administering to a patient in need of such
treatment an effective amount of a peptidomimetic
according to claim 1 or 13.

        23.   A peptidomimetic according to claim 1
wherein β is a residue of an aminobenzoic acid.

15   24.   A peptidomimetic according to claim 23
wherein X is leucine or isoleucine.

        25.   A peptidomimetic according to claim 24
wherein β is a substituted 4-aminobenzoic acid.

        26.   A peptidomimetic according to claim 25
20 wherein the cysteinyl moiety is in the reduced
state.

        27.   A peptidomimetic according to claim 26
wherein the 4-aminobenzoic acid is substituted at
the 2- and/or 3- position of the phenyl ring by an
25 alkyl, alkoxy, aryl, or naphthyl group, or a
heterocyclic or heteroaromatic ring.

        28.   A peptidomimetic according to claim 26
wherein β is 2-phenyl-4-aminobenzoic acid or 2-
naphthyl-4-aminobenzoic acid.

29.   A peptidomimetic according to claim 28
wherein L is leucine.

30.   A pharmaceutical composition comprising
a peptidomimetic according to claim 24 and a
pharmaceutically acceptable carrier.

31.   A method of inhibiting
geranylgeranyltransferase in a host where the
geranylgeranyltransferase is present which
comprises administering to the host an effective
amount of a peptidomimetic according to claim 24.

32.   A peptidomimetic according to claim 24
in the form of a pro-drug.

33.   A pro-drug according to claim 32 wherein
the pro-drug comprises a compound as defined with
one or more terminal amino, sulfhydryl and acid
groups functionalized with a lipophilic, esterase-
sensitive moiety.

34.   A pro-drug according to claim 33 wherein
a terminal carboxylic acid group is esterified.

35.   A pro-drug according to claim 34 wherein
the carboxylic acid group is esterified as the
methyl ester.

36.   A method of inhibiting
geranylgeranyltransferase in a host where
geranylgeranyltransferase is present which
comprises administering to the host an effective
amount of the compound of claim 35.

37.   A method of treating cancer comprising
administering to a patient in need of such

treatment an effective amount of a peptidomimetic
according to claim 24.

5

38.   A method of treating cancer comprising
administering to a patient in need of such
treatment an effective amount of a peptidomimetic
according to claim 35.

39.   A compound of the formula

$$C^0B$$

wherein

10

C^0 stands for

$$R_0-CH_2-CH\overset{\overset{A}{\|}}{-}C-$$
$$\underset{NH_2}{|}$$
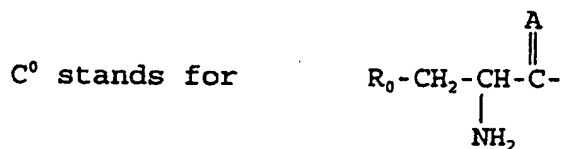
15   A representing O or 2H, and
          R_0 representing SH, NH_2, or $C_xH_y-SO_2-NH-$,
wherein C_xH_y is a straight chain saturated or
unsaturated hydrocarbon, with x being between 1
and 20 and y between 3 and 41, inclusive; and

20   B stands for -NHR, wherein R is an aryl group.

40.   A compound according to claim 39 wherein
R is a biphenyl group.

41.   A compound according to claim 39 wherein
C^0 is 3-mercapto-2-amino-propylamino.

25   42.   A compound according to claim 41 wherein
R is a biphenyl group.

43.   A compound according to claim 42 wherein
R is a biphenyl group substituted with -COOH.

44.   A compound according to claim 42 wherein
R is a biphenyl group substituted with lower alkyl
or oxyalkyl.

45.   A compound according to claim 44 wherein
the lower alkyl is methyl.

46.   A compound according to claim 39 of the
formula:
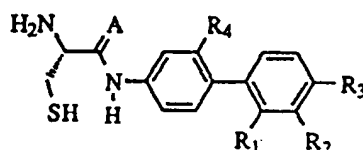
wherein A is O or 2H; R_1 is hydrogen or COOH; R_2 is
hydrogen, COOH, $COOCH_3$, CH_3 or tetrazolyl; R_3 is
hydrogen or COOH; and R_4 is hydrogen, OCH_3, OC_3H_7, or
a phenyl group.

47.   A compound according to claim 46 wherein
R_1 is hydrogen; R_2 is hydrogen, COOH or CH_3; R_3 is
hydrogen or COOH, R_2 being COOH or CH_3 when R_3 is
hydrogen; and R_4 is hydrogen.

48.   A compound according to claim 47 wherein
R_2 is COOH and R_3 is hydrogen.

49.   A compound according to claim 46 wherein
R_1 and R_3 are H, R_2 is COOH or CH_3, and R_4 is a
phenyl or n-oxypropyl group.

50.   A compound according to claim 46 wherein
R_1, R_3, and R_4 are H; and R_2 is tetrazolyl.

51. A compound according to claim 40,
wherein R_0 is C_xH_y-SO_2-NH-, x is 2-16 and y is 3 to
33.

52. A compound according to claim 51,
wherein R_0 is selected from the group consisting of
C_2H_5-SO_2-NH-, CH_2=CH-SO_2-NH-, and $CH_3(CH_2)_{15}$-SO_2-NH-.

53. A compound according to claim 40,
wherein R_0 is NH_2-.

54. A compound according to claim 53,
wherein R is a 2'carboxy-biphenyl group or 1
methoxy-2'-carboxy-biphenyl group.

55. A compound according to claim 39 wherein
R_0 is SH and B is 3 aminomethyl-3'-carboxy-
biphenyl.

56. A method of inhibiting p21ras
farnesyltransferase in a host in need of such
inhibition which comprises administering to said
host an effective amount of a compound according
to claim 39.

57. A pharmaceutical composition comprising
a compound according to claim 39 and a
pharmaceutically acceptable carrier therefor.

58. A method of inhibiting p21ras
farnesyltransferase in a host in need of such
inhibition which comprises administering to said
host an effective amount of a compound according
to claim 41.

59.   A pharmaceutical composition comprising
a compound according to claim 41 and a
pharmaceutically acceptable carrier therefor.

60.   A peptidomimetic of formula:

5   CβX

wherein
      C represents cysteine;
      X   is an amino acid; and
      β   is a non-peptide aminoalkyl- or amino-
10   substituted aliphatic or aromatic
             carboxylic acid or a heterocyclic
             monocarboxylic acid.

61.   A peptidomimetic according to claim 60
wherein β is an aminobenzoic or aminoalkanoic
15   acid.

62.   A peptidomimetic of the formula:
                           CΔ
wherein C is cysteine and Δ is an aryl or
heterocyclic substituent which does not include a
20   peptide amino acid but corresponds essentially in
      size with a tetrapeptide of the formula CA_1A_2X
      wherein C is cysteine and A_1, A_2 and X are peptide
      amino acids.

63.   A peptidomimetic according to claim 65
25   comprising cysteine joined directly to an
      aminomethyl-bicyclic arylcarboxylic acid.

64.   A compound according to claim 63 wherein
Δ comprises a biphenyl group.

65. A compound according to claim 64 wherein
Δ is the radical of 3-aminomethyl-biphenyl-3-
carboxylic acid.

66. A compound of the formula:

$$\Delta_1 \; C \; \Delta \; ,$$
$$\Delta_1 \; CA_1A_2 \; X$$

or

$$\Delta_1 \; C\text{-}\beta\text{-}X$$

wherein

C is cysteine;

A_1, A_2 and X are peptide amino acids;

Δ   is an aryl or heterocyclic
        substituent which does not include
        a peptide amino acid;

β   is a non-peptide aminoalkyl- or
        amino-substituted aliphatic or
        aromatic carboxylic acid or
        hetercarboxylic acid; and

Δ_1 is a phosphonate group joined to C
        through the cysteine S atom.

67. A compound according to claim 66 of the
formula:

$$\Delta_1 \; C\text{-}AMBA\text{-}X$$

wherein Δ_1, C and X have the meanings given in
claim 56 and AMBA is an aminomethyl-benzoic acid.

68. A compound according to claim 67 wherein
AMBA is 3-aminomethyl benzoic acid.

69. A compound of the formula:
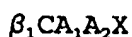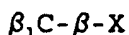
$$\beta_1 C\Delta \; ,$$
$$\beta_1 CA_1A_2 X$$

or

$$\beta_1 C\text{-}\beta\text{-}X$$

wherein

      C is cysteine; A_1, A_2 and X are peptide amino acids;

      Δ   is an aryl or heterocyclic substituent which does not include a peptide amino acid;

      β   is a non-peptide aminoalkyl- or amino-substituted aliphatic or aromatic carboxylic acid or heterocarboxylic acid; and

      $β_1$ is an aryl or aralkyl group joined to C through the cysteine S atom.

    70.   A compound according to claim 69 of the formula:

$$β_1 \text{ C-AMBA-X}$$

wherein C, X and $β_1$ have the meanings given in claim 70 and AMBA is an aminomethyl benzoic acid.

    71.   A compound according to claim 70 wherein AMBA is 3-aminomethylbenzoic acid.

72.   A compound of the formula:
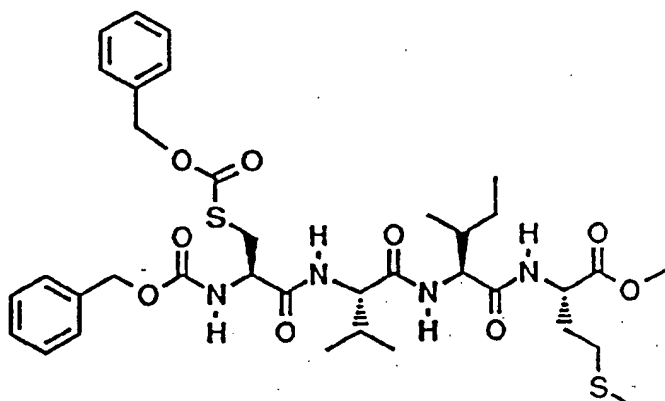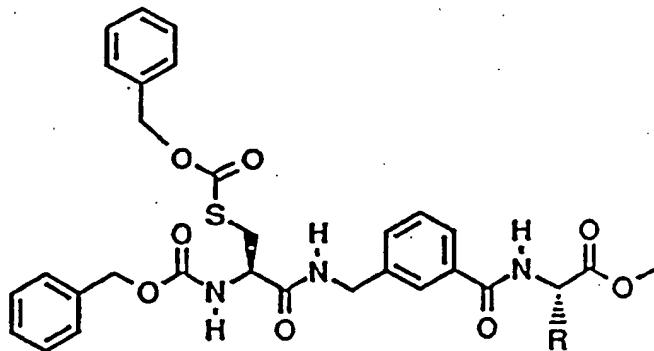
or
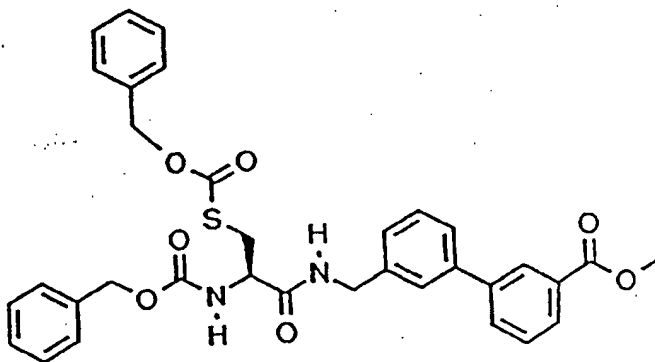
73. A method of improving peptide or
peptidomimetic uptake into cells which comprises
utilizing the peptide in a functionalized form
such that terminal amino, sulfhydryl and acid
5   groups are functionalized by lipophilic or
hydrophobic, esterase-sensitive moieties.

74. A method according to claim 73 for
inhibiting tumor cell growth in a host which
comprises administering to the host an effective
10   amount of a peptide or peptidomimetic wherein one
or more terminal amino, sulfhydryl and carboxylic
acid groups have been functionalized with a
lipophilic or hydrophobic esterase-sensitive
moiety.

15   75. A method according to claim 74 wherein
the terminal amino and sulfhydryl groups are
functionalized by benzyloxy carbonyl groups and
the carboxylic acid group is esterified.

76.   A compound represented by one of
formulas (A-L):

wherein R_1' is

i)   hydrogen;

ii)   lower alkyl;

iii)   alkenyl;

iv)   alkoxy;

v)   thioalkoxy;

vi)   halo;

vii)   haloalkyl;

viii)   aryl-L_2-, wherein L_2 is absent, -CH_2-, -CH_2CH_2-, -$CH(CH_3)$-, -O-, -$S(O)_q$ wherein q is 0, 1, or 2, -$N(R')$- wherein R' is hydrogen or lower alkyl, or -$C(O)$- and aryl is selected from the group consisting of phenyl, naphthyl, tetrahydronaphthyl, indanyl and indenyl and the aryl group is unsubstituted or substituted; or

ix)   heterocyclic-L_3- wherein L_3 is absent, -CH_2-, -CH_2CH_2-, -$CH(CH_3)$-, -O-, -$S(O)_q$ wherein q is 0, 1 or 2, -$N(R')$- wherein R' is hydrogen or loweralkyl, or -$C(O)$- and heterocyclic is a monocyclic heterocyclic wherein the heterocyclic is unsubstituted or substituted with one, two, or three substituents independently selected from the group consisting of loweralkyl, hydroxy, hydroxyalkyl, halo, nitro, oxo (=O), amino, N-protected amino, alkoxy, thioalkoxy and haloalkyl;

R_{1a} is hydrogen or lower alkyl;

R_2' is

i)

wherein R_{12a} is hydrogen, loweralkyl or -$C(O)O$-R_{13}, wherein R_{13} is hydrogen or a carboxy-protecting

group and R_{12b} is hydrogen or loweralkyl, with the proviso that R_{12a} and R_{12b} are not both hydrogen,

ii) $-C(O)NH-CH(R_{14})-C(O)OR_{15}$ wherein R_{14} is
a)   loweralkyl,
b)   cycloalkyl,
c)   cycloalkylalkyl,
d)   alkoxyalkyl,
e)   thioalkoxyalkyl,
f)   hydroxyalkyl,
g)   aminoalkyl,
h)   carboxyalkyl,
i)   alkoxycarbonylalkyl,
j)   arylalkyl or
k)   alkylsulfonylalkyl and

R_{15} is hydrogen or a carboxy-protecting group or

iii)

$$- C(O) - HN - \overset{\displaystyle O}{\underset{(CH_2)_{1\text{-}3}}{\bigcirc}} \quad ;$$

R_3' is

$$R_0-CH_2-CH-\overset{\displaystyle A}{\underset{NH_2}{\overset{\|}{C}}}-$$

where

       A represents O or 2H, and
       R_0 represents SH, NH_2, or $C_xH_y-SO_2-NH-$, wherein
C_xH_y is a straight chain saturated or unsaturated

hydrocarbon, with x being between 1 and 20 and y
between 3 and 41, inclusive; and
B stands for -NHR, where R is an aryl group.
and

5   L_1 is -NH-;

or pharmaceutically acceptable salts or prodrugs
thereof.

1 / 22

FIG. IA

CVIM

FTI - 249

FTI - 276:   R = O⁻
FTI - 277:   R = OCH₃

FIG. IB

log M FTI—276

RasF

	nM					μM			
FTI-277	0	10	30	100	300	1	3	10	50

Ras $\begin{smallmatrix}U\\P\end{smallmatrix}$

Rap1A $\begin{smallmatrix}U\\P\end{smallmatrix}$

FIG. 2A

	pZIPneo		RasF		RasGG		Raf		S186	
FTI-277	-	+	-	+	-	+	-	+	-	+

Ras $\begin{smallmatrix}U\\P\end{smallmatrix}$　　　　　　　　　　　　　　　　　　　3 sec

Ras $\begin{smallmatrix}U\\P\end{smallmatrix}$　　　　　　　　　　　　　　　　　　　45sec
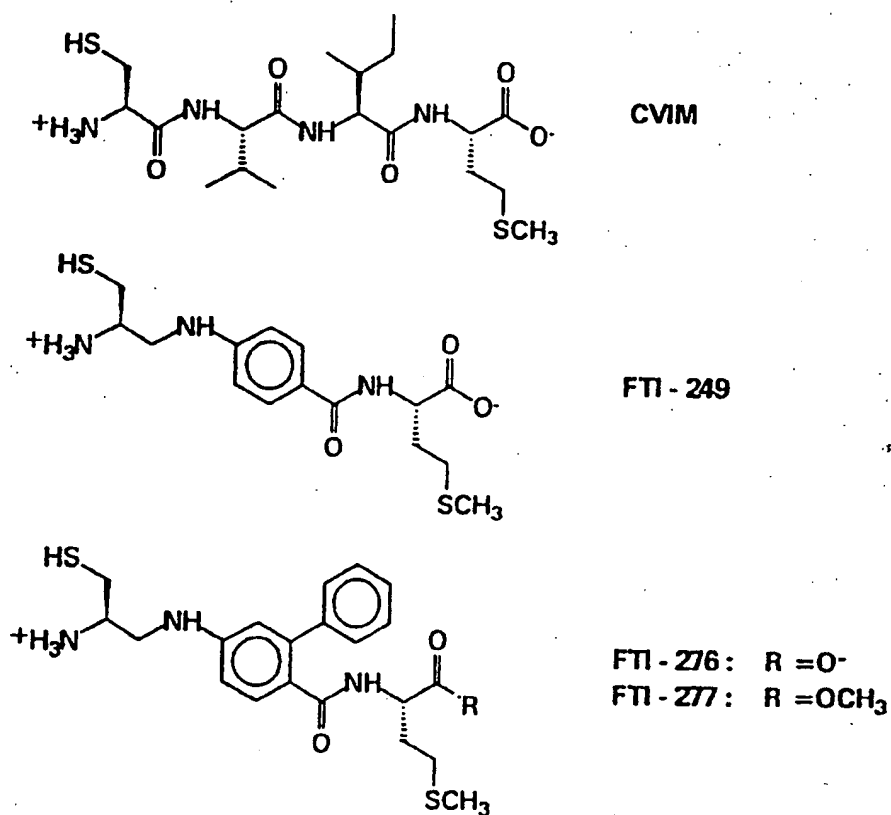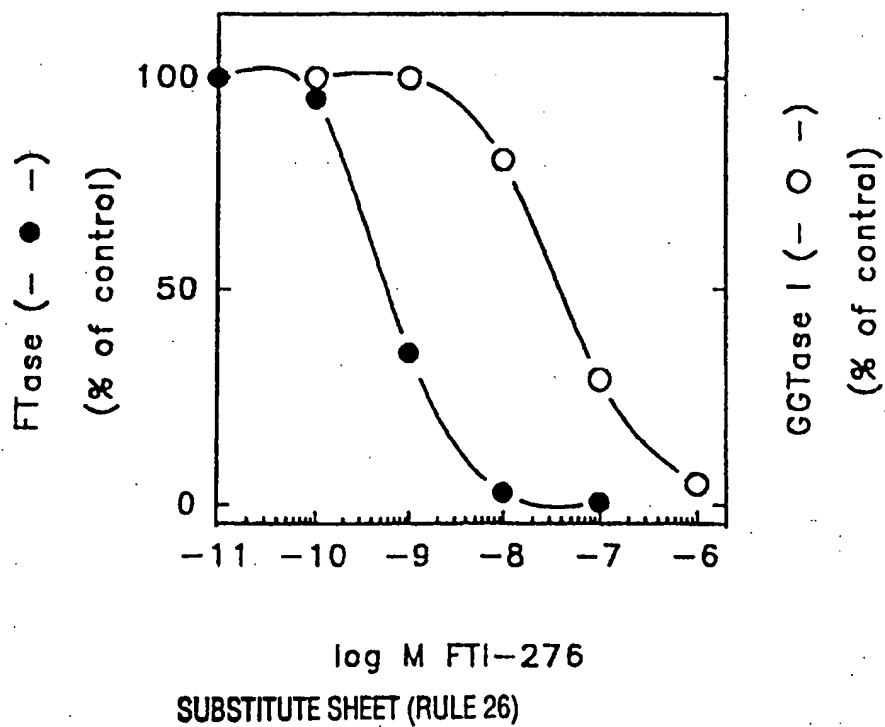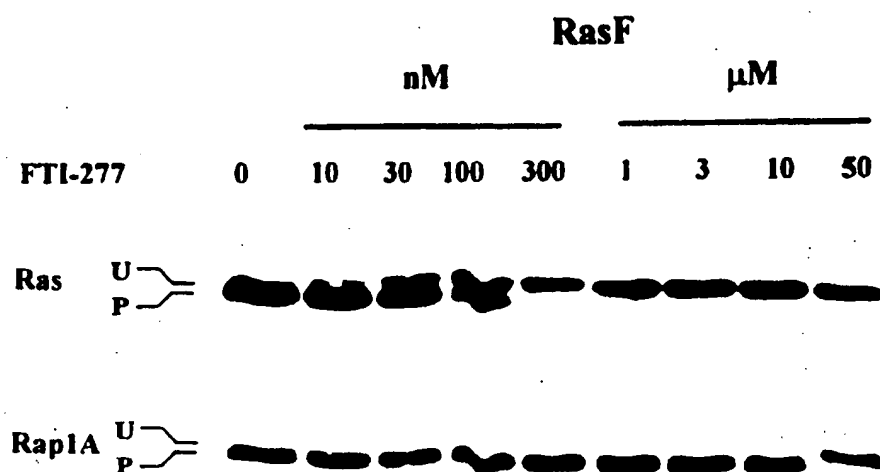
FIG. 2B
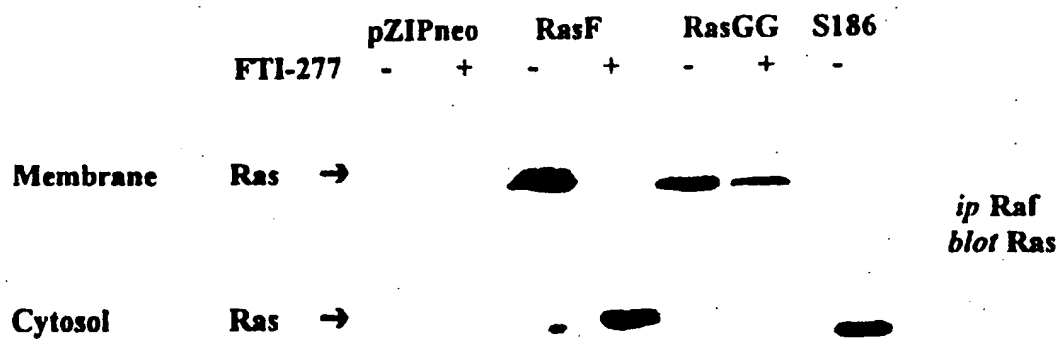
FIG. 3
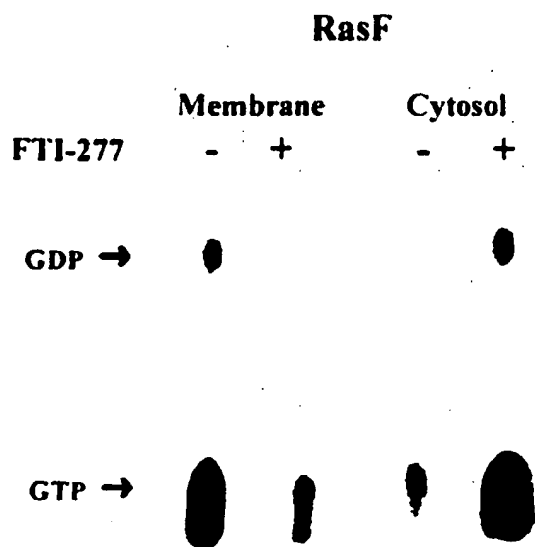
FIG. 4A
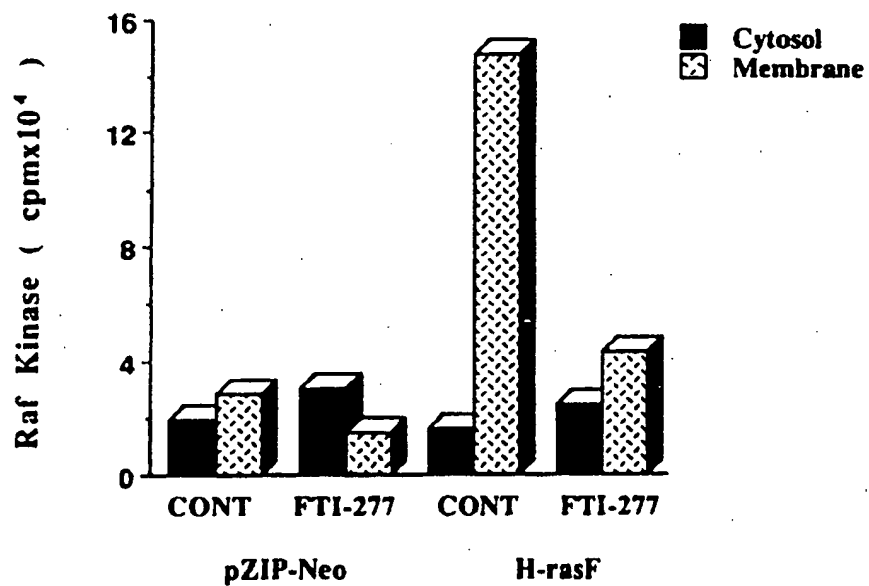
FIG. 4B SUBSTITUTE SHEET (RULE 26)

5 / 22
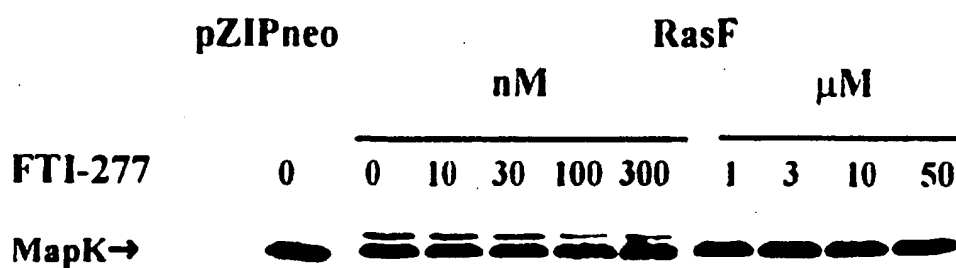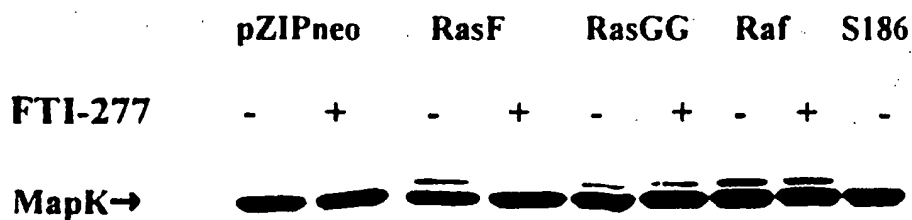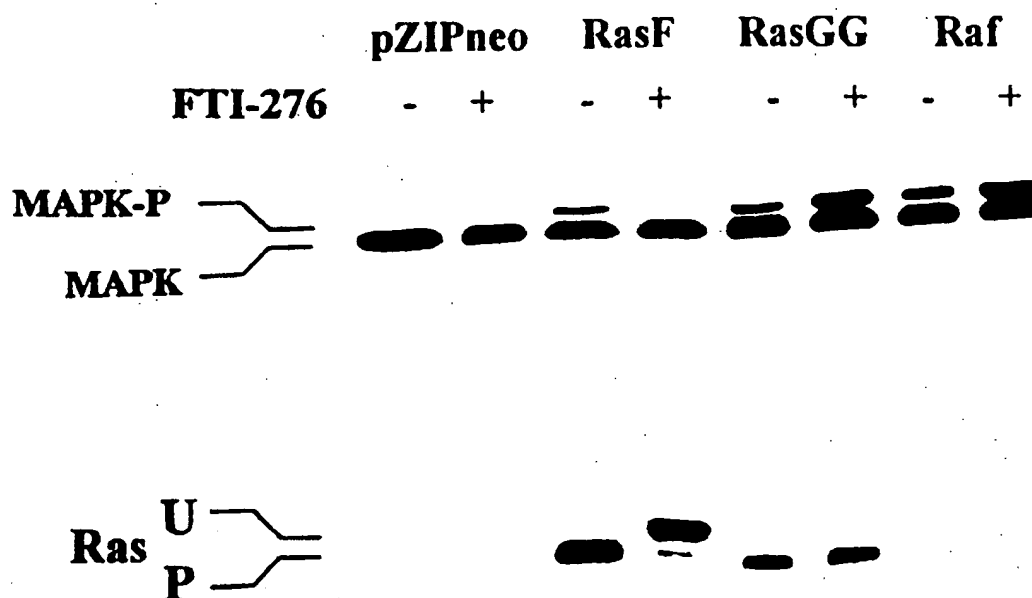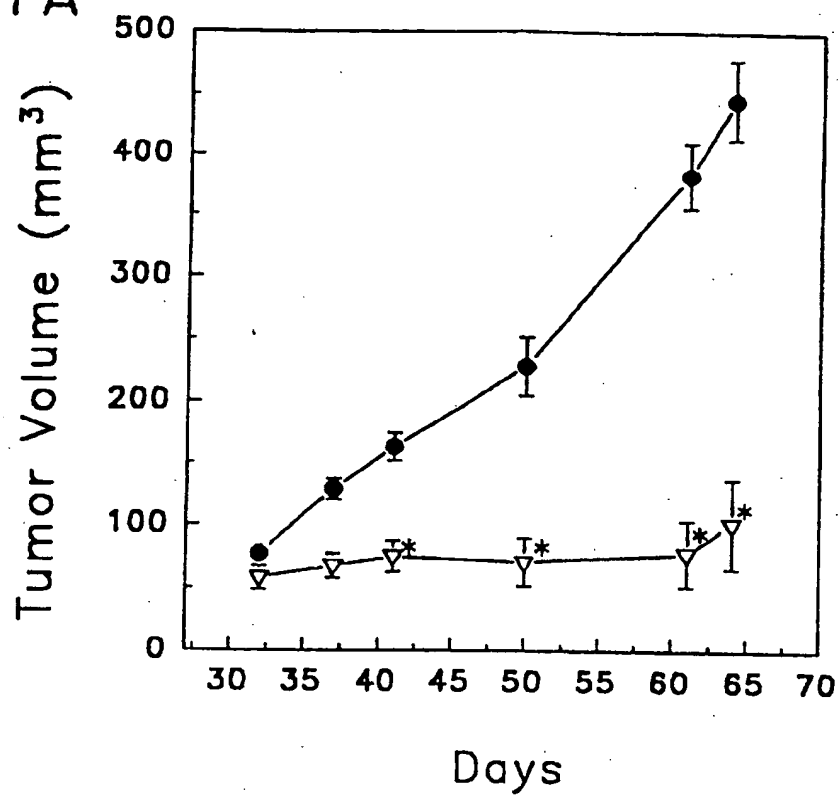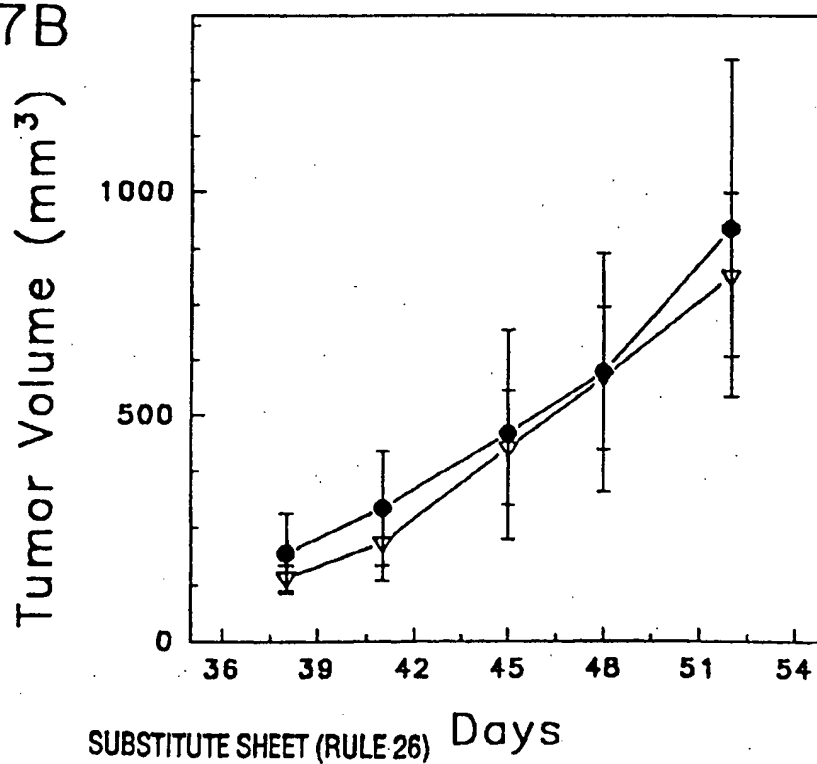
	pZIPneo		RasF								
			nM						μM		
FTI-277	0	0	10	30	100	300		1	3	10	50
MapK→											

FIG. 5A

	pZIPneo		RasF		RasGG		Raf		S186
FTI-277	-	+	-	+	-	+	-	+	-
MapK→									

FIG. 5B

FIG. 6

FIG. 7A

FIG. 7B

FIG. 8A

FIG. 8B

FIG. 9A

FIG. 9B

RAS

FIG. IOA

FIG. IOB

FIG. 11A

FTI-276 (mg/kg)   0   10   50   100

Ras

Rap1A

FIG. 11B

FIG. 12

CVIM

1

C-4ABA-M

2

REDUCED
C-4ABA-M

2 a
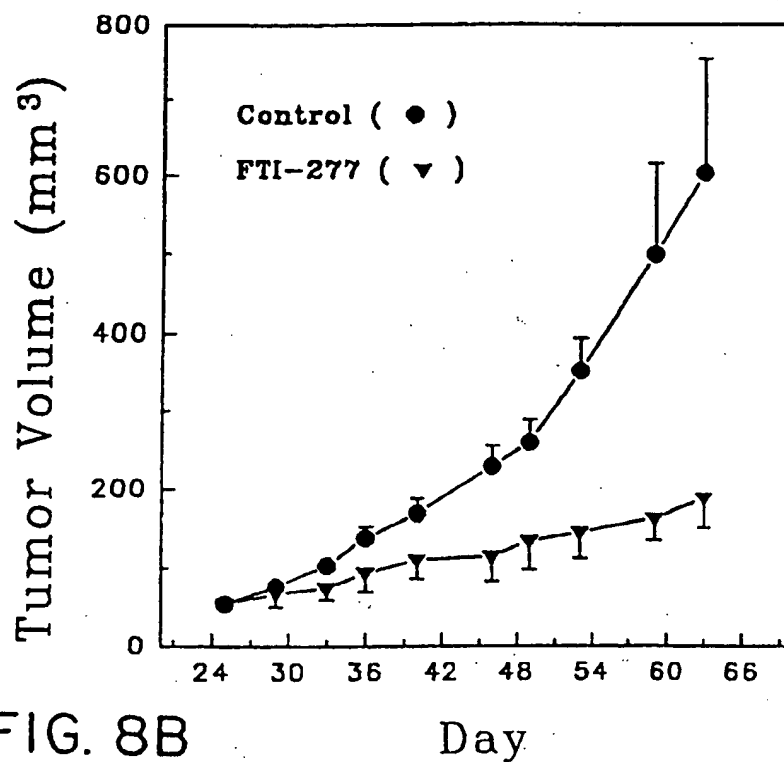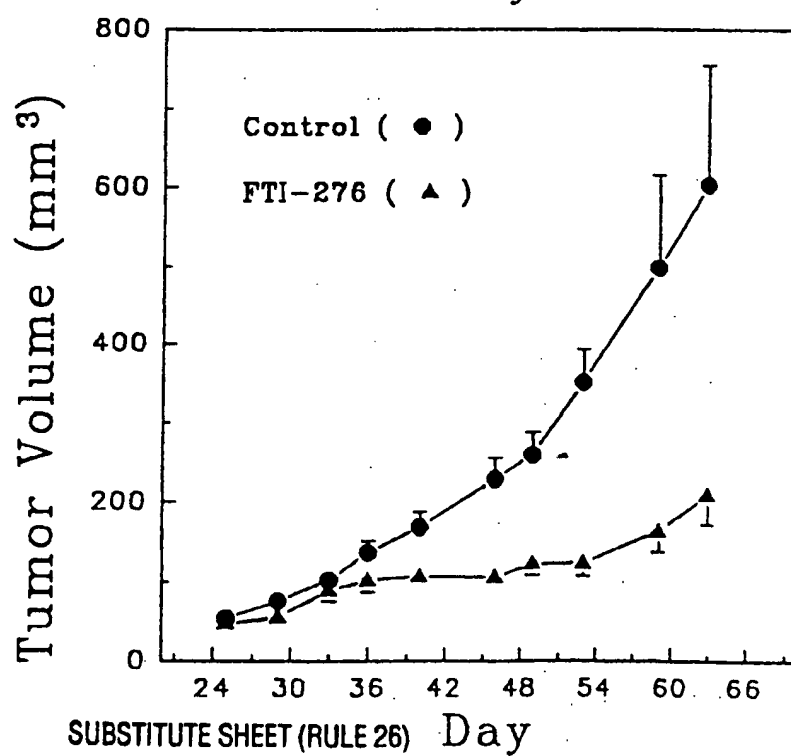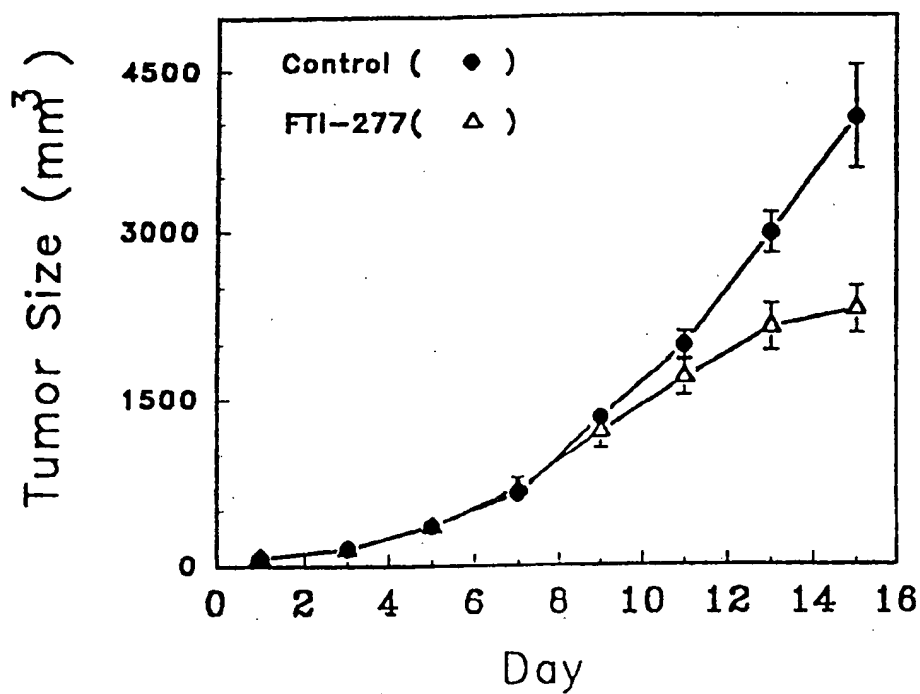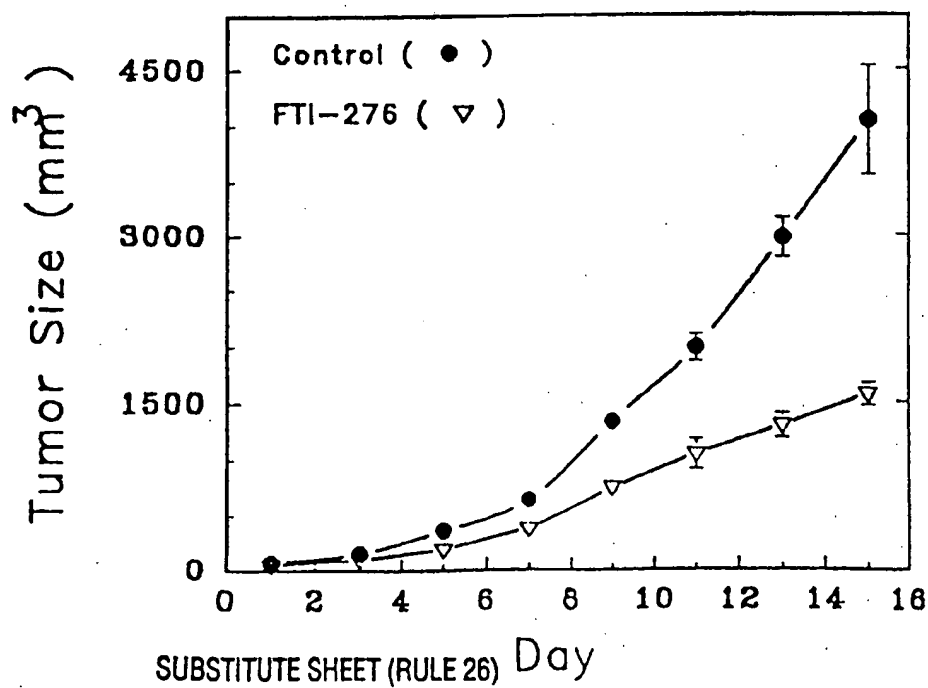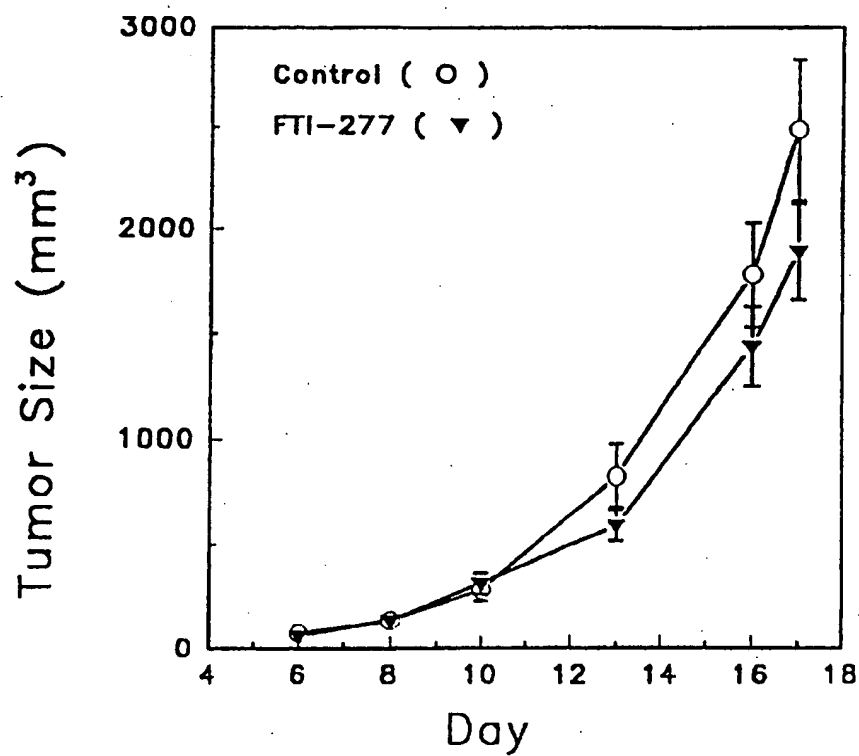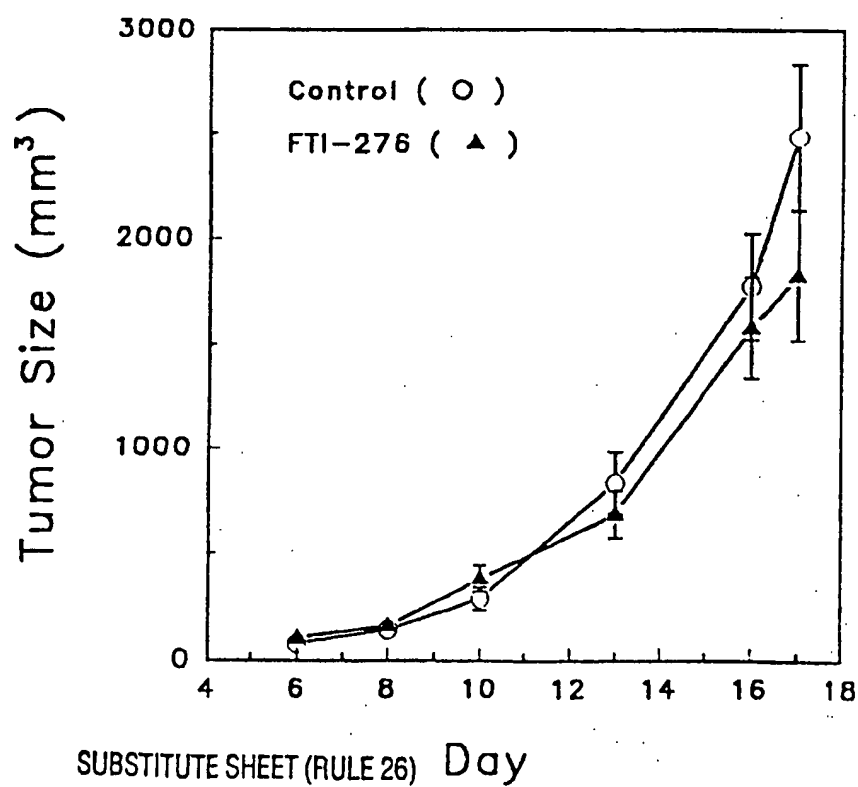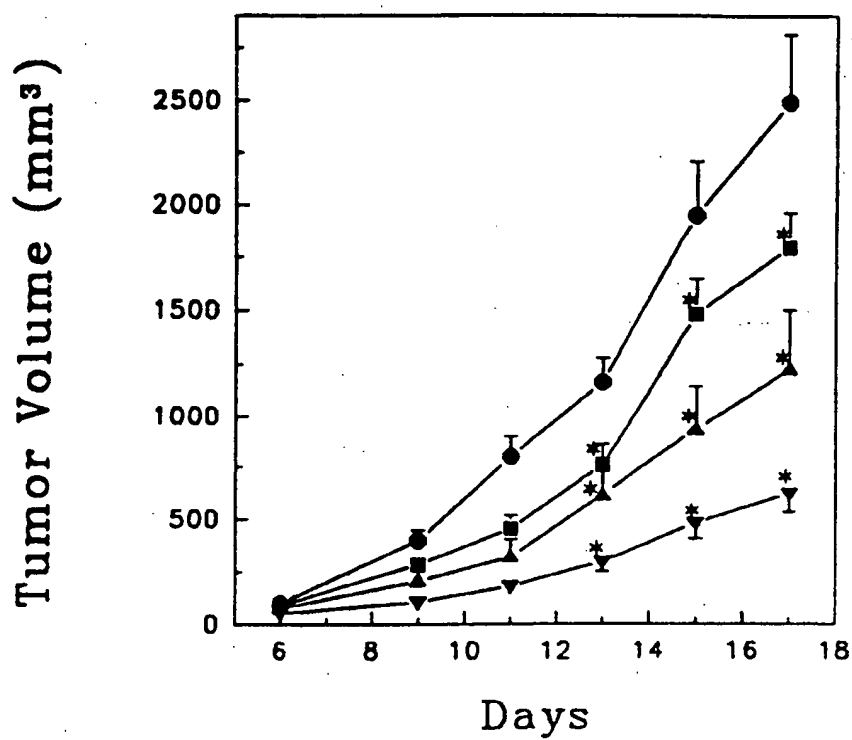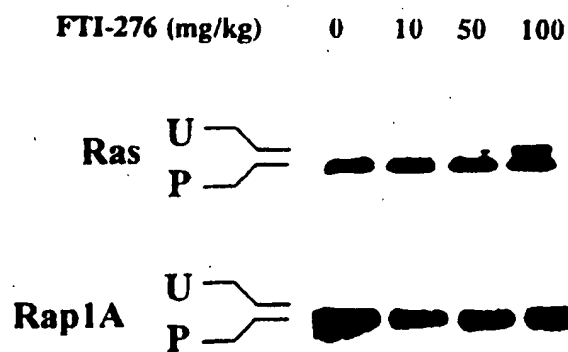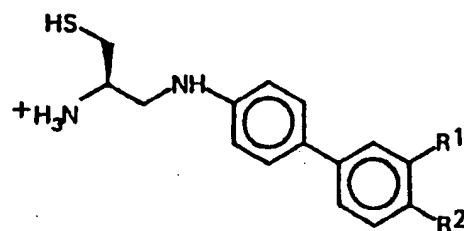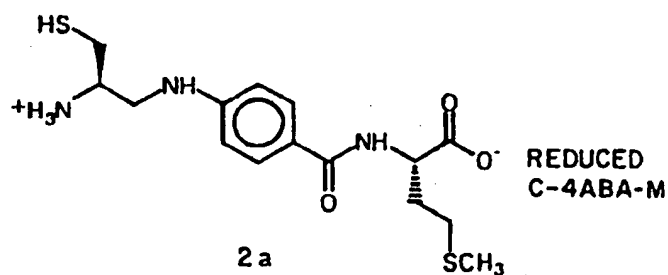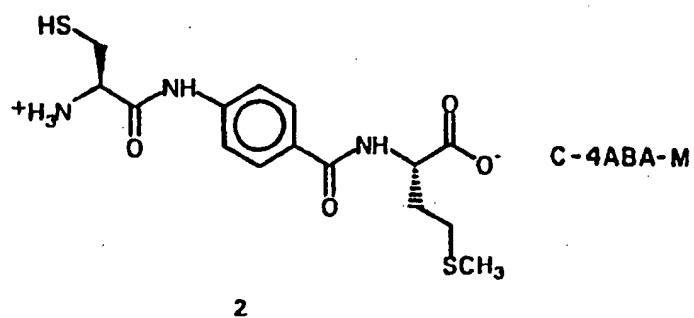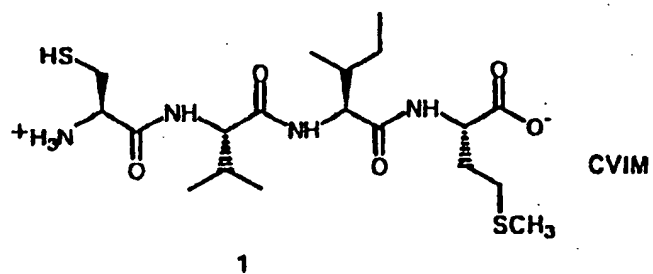
4	R^1=COOH	R^2=H
5	R^1=H	R^2=COOH
8	R^1=CH$_3$	R^2=H

FIG. 13

FIG. 14A

FIG. 14B

F-OH

F-peptide

FPP

Origin

1 2 3 4 5 6 7 8 9
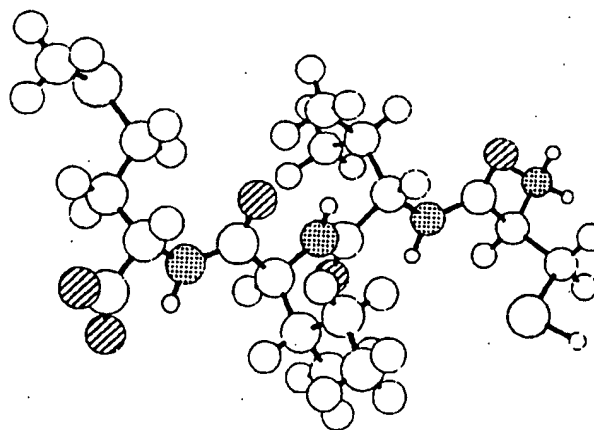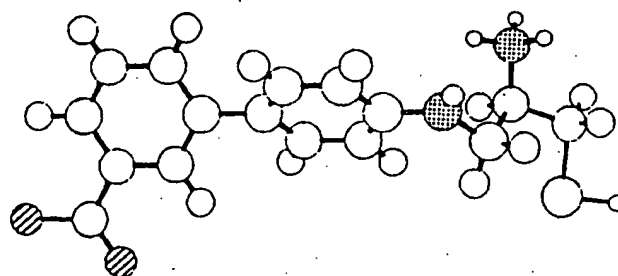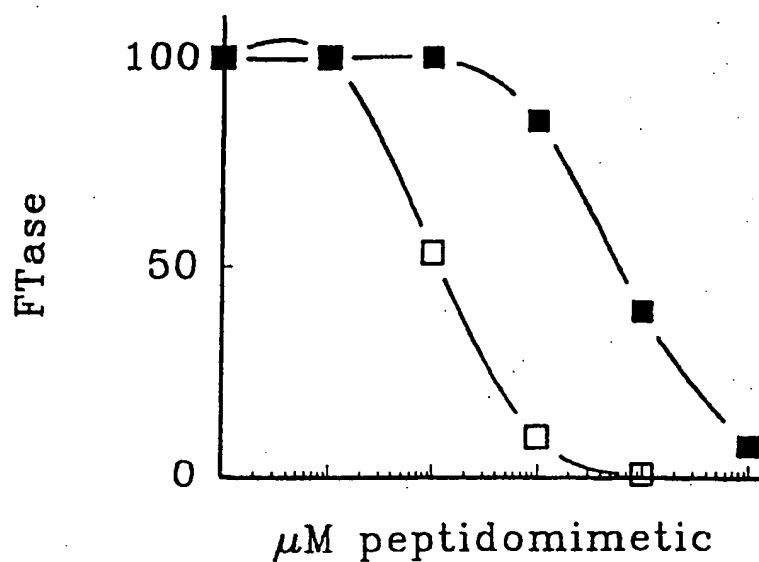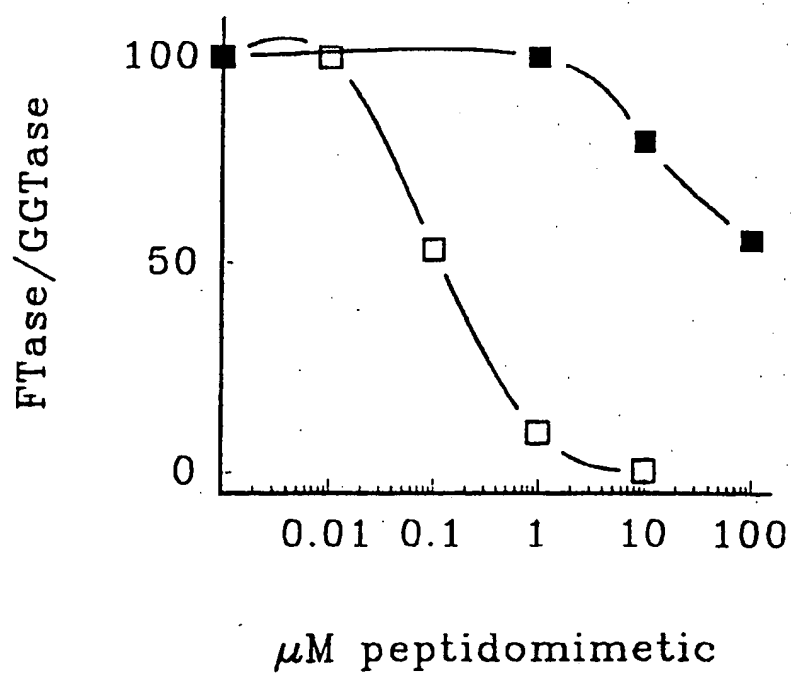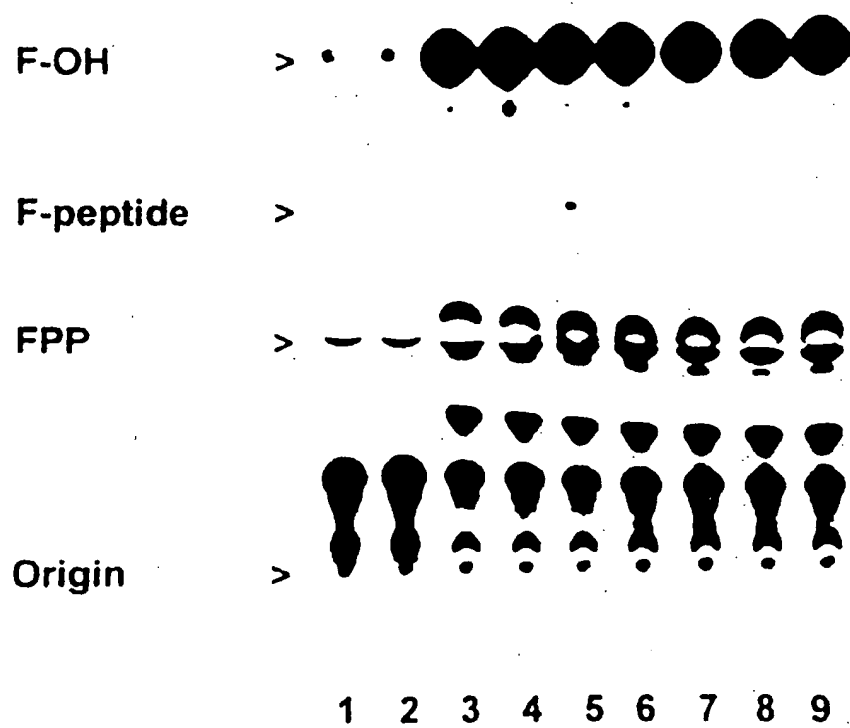
FIG. 15

Ras
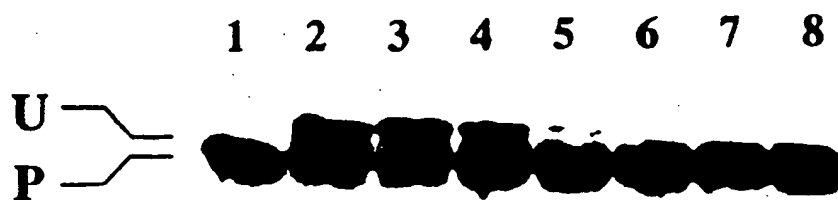
FIG. 16A

Rap1A
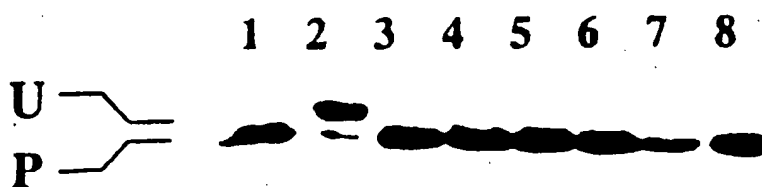
FIG. 16B

17 / 22
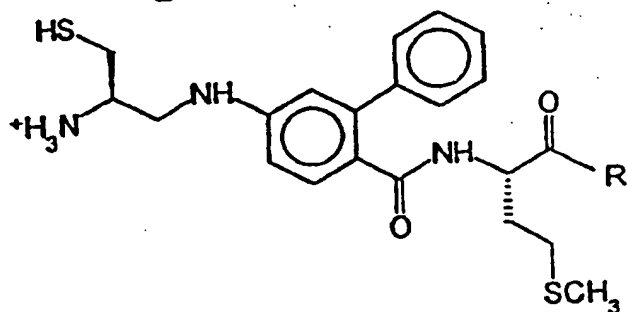
FIG. 17

FTI-276: R = O⁻
FTI-277: R = OCH₃
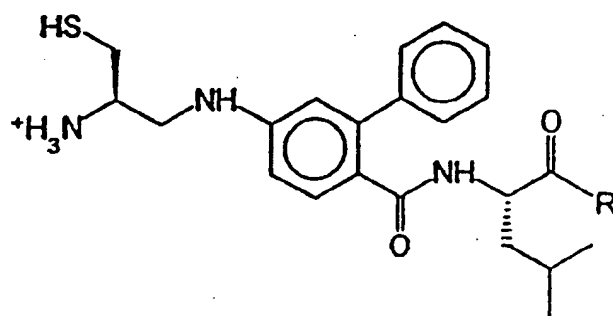
GGTI-286: R = OCH₃
GGTI-287: R = O⁻

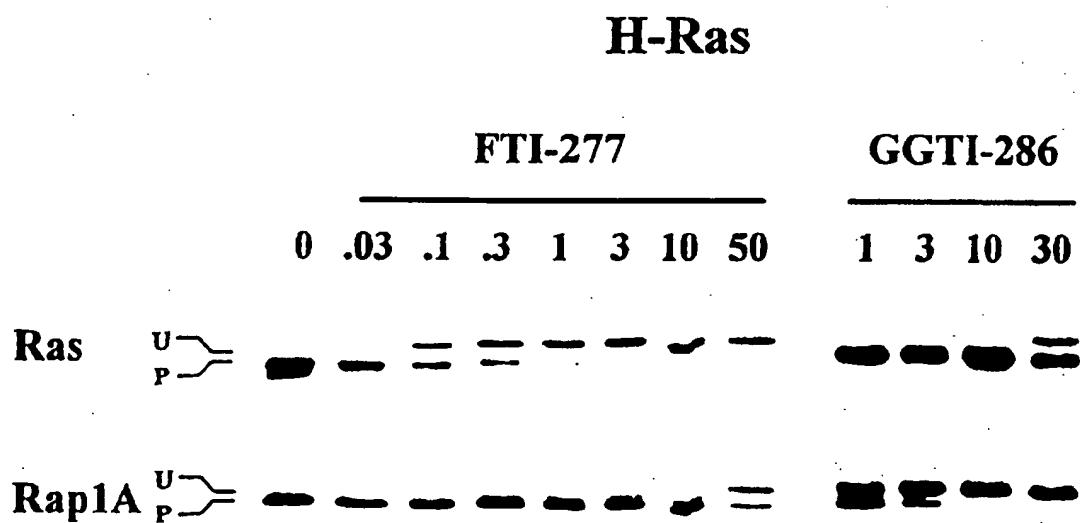GGTI-297: red. 2-(1-Naphthyl) CABAL
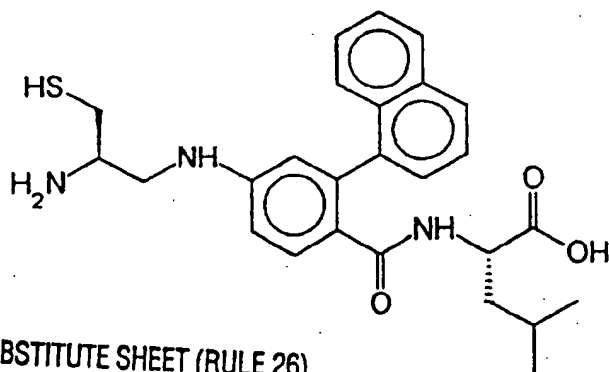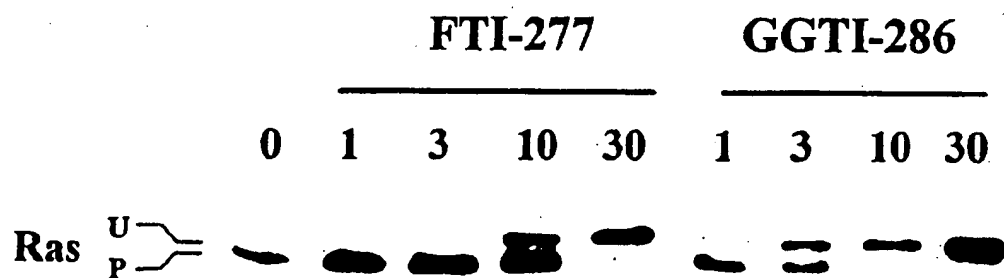
SUBSTITUTE SHEET (RULE 26)
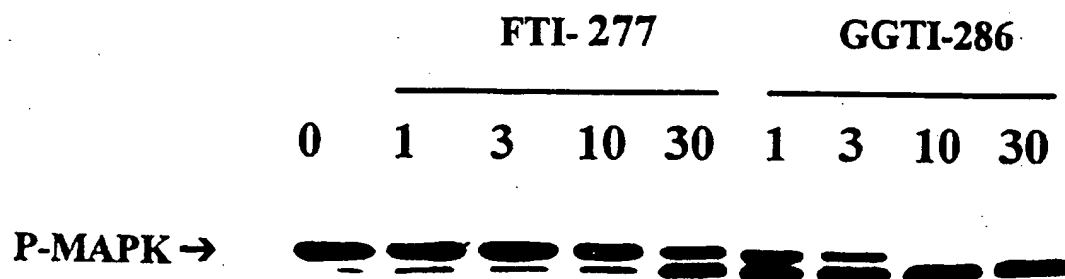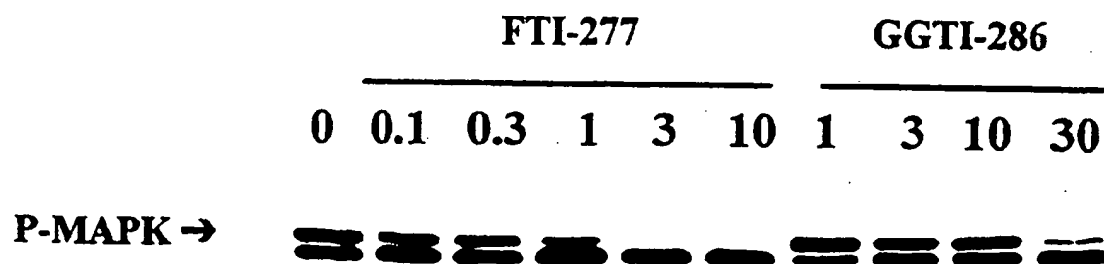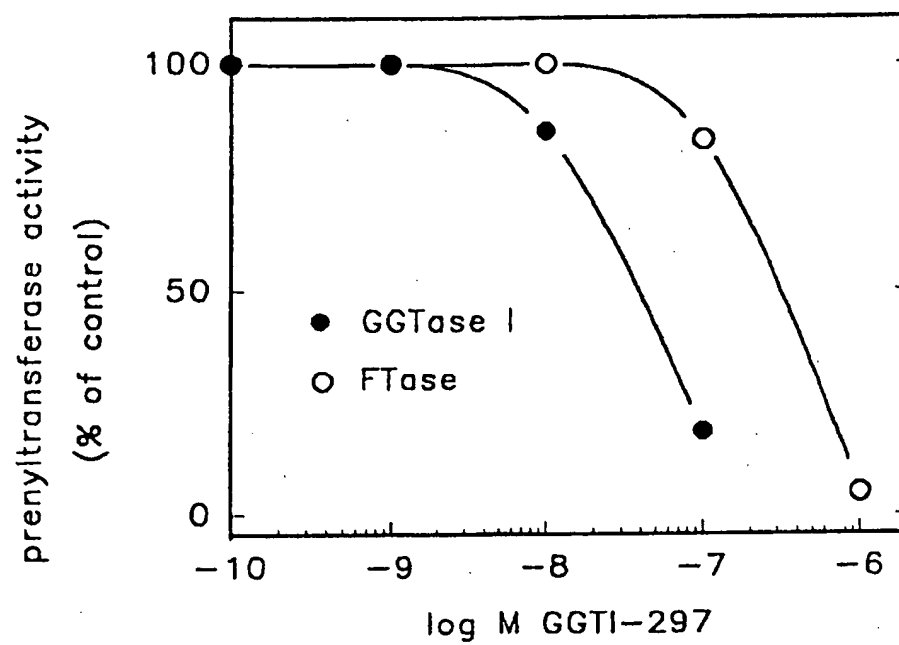
FIG. 18

K-Ras4B

FTI-277   **GGTI-286**

0   1   3   10   30   1   3   10   30

Ras U⎤⎱
    P ⎦

FIG. 19

K-Ras4B

	FTI- 277				GGTI-286			
0	1	3	10	30	1	3	10	30

P-MAPK →

H-Ras

	FTI-277					GGTI-286			
0	0.1	0.3	1	3	10	1	3	10	30

P-MAPK →

FIG. 20

FIG. 21

FIG. 22

Antitumor Efficacy in K−ras4B
GGTI-286

- ● 0 mg 1Kg
- ▲ 25 mg 1Kg
- ▼ 50 mg IKg
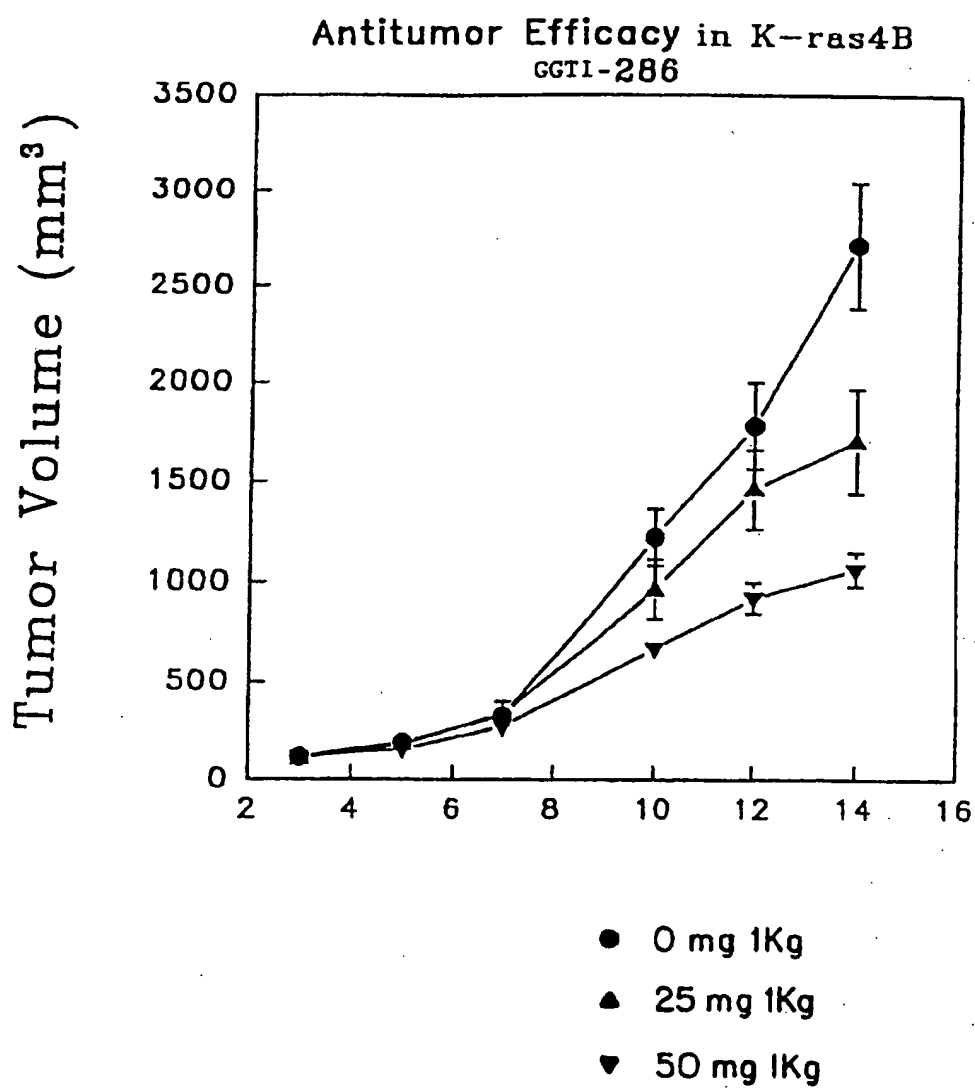
A. CLASSIFICATION OF SUBJECT MATTER		

IPC(6) :A61K 38/06
US CL :514/18
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED		

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/18, 19; 562/557

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS Online, APS

C. DOCUMENTS CONSIDERED TO BE RELEVANT		

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Cell, Volume 62, issued 13 July 1990, Y. Reiss et al, "Inhibition of Purified p21'ᵃˢ Farnesyl:Protein Transferase by Cys-AAX Tetrapeptides", pages 81-88, see entire document.	1-76

☐ Further documents are listed in the continuation of Box C.   ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance		
"E"	earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
31 MAY 1996	21 JUN 1996

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	DAVID LUKTON
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

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